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Delirium and long-term cognitive impairment after stroke: the role of the hypothalamic-pituitary-adrenal axis

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This thesis is dedicated to the memory of my late father, Ronald L Barugh

Contents

Declaration

The research described in this thesis was the unaided work of the author, except where acknowledgement is made by reference. No part of this work has previously been accepted for any other degree, nor is any part of it being concurrently submitted in candidature for another degree.

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Abstract

Delirium is a severe neuropsychiatric syndrome, characterised by the acute onset of inattention, altered level of arousal, and other mental status abnormalities. Delirium is extremely common in acute stroke, affecting at least 1 in 5 such patients admitted to hospital. It is a serious complication of stroke, being associated with higher mortality, longer length of hospital stay and higher dependency at discharge. The pathophysiology of delirium is not completely understood, and there are no specific treatments. This thesis investigated the role of cortisol in the development of delirium after stroke and also investigated the role of delirium and of cortisol in the development of cognitive impairment in the 12 months after stroke. The thesis specifically investigated whether levels of cortisol in saliva are elevated in delirium and also whether there is a loss of the normal diurnal rhythm in delirium, evidenced by elevated afternoon salivary cortisol levels and reduced morning level to afternoon level ratio. The thesis also investigated whether cortisol levels are persistently elevated in the year after stroke in those who developed delirium and whether cortisol levels are associated with cognitive decline. Finally it investigated whether acute and/or chronic changes seen on Computed Tomography (CT) brain scans taken around the time of stroke onset are associated with the development of delirium after stroke

A longitudinal cohort study was conducted in 95 participants aged 60 years or over, who were admitted to hospital with a clinically confirmed stroke. Participants gave informed consent, or proxy consent was obtained if they lacked capacity to consent. At baseline participants underwent brief cognitive testing and were then assessed for the presence of delirium, using DSM IV criteria, at regular intervals during the first two weeks after stroke. At each assessment a saliva sample was collected in the morning and in the afternoon, to measure cortisol. Participants were then visited at 1 month, 4 months and 12 months after stroke onset, at which point they were assessed for the presence of delirium, further saliva samples were taken and a cognitive test battery was completed.

26 (27%) participants developed delirium during the course of the study period. The study found elevated salivary cortisol levels in those with delirium at up to 4 months after stroke, but at 12 months there was no difference between the delirium and no delirium group. A loss of the diurnal rhythm was seen in those who developed delirium at 5 days after stroke, but the diurnal variation had returned to a normal pattern at follow-up. However, in a multivariate analysis, controlling for age, sex, stroke severity (NIHSS), current illness burden (APACHE

II), chronic illness burden (CCI) and prior cognitive impairment (IQCODE), neither median salivary cortisol levels in the first two weeks after stroke, nor the ratio of morning to afternoon cortisol levels were independent predictors of delirium diagnosis, although median 9am cortisol approached significance (OR=0.95, 95% confidence interval (CI) 0.89-1.01, p=0.08). In a random effects logistic regression analysis, the probability of developing delirium decreased over time from stroke onset and increased per unit increase in salivary cortisol (nmol/L), however this effect was not statistically significant (OR 1.02, CI 0.84-1.19 P=0.70 for morning cortisol and OR 1.05, CI 0.82-1.25 p=0.46 for afternoon cortisol). Global cognition, measured by the MoCA, was significantly poorer in the delirium group at each time point throughout the 12 months after stroke. However, there was a trend towards improvement in MoCA scores in the whole cohort throughout the 12 month follow-up, with the exception of those who developed the most severe delirium. The presence of delirium at any point during the 12 month follow-up did not affect the rate of change of the MoCA scores over the 12 months after stroke. The presence of brain atrophy identified on admission CT brain scans was independently associated with delirium (OR 3.7, CI 1.15-11.88, p=0.02), however the presence of a visible acute or chronic stroke lesion and the presence of white matter lesions were not. Finally, those who developed delirium had a worse functional outcome, longer length of hospital stay and were more likely to require institutional care or a package of care at home, compared with those who did not develop delirium.

This thesis has contributed to our understanding of the mechanisms and phenomenology of delirium after stroke, and has also highlighted areas for further research which will be required to unpick the complex pathophysiology of delirium.

Lay summary

Delirium, or "acute confusional state" is common after stroke and has serious adverse consequences including longer length of stay in hospital, a higher risk of being discharged to an institution (rather than home) and a higher risk of death. The underlying mechanisms which cause delirium are not well understood and consequently there are currently no specific treatments available. The studies presented in this thesis investigated whether the stress hormone cortisol is found in higher levels in those with delirium after a stroke, when compared to those without delirium after stroke. The studies also investigated the duration of delirium, by following participants up over the course of a year, and investigated whether cortisol levels remained high over that year in those who had delirium. The studies also investigated the relationship between cognitive function and delirium over the course of a year, and whether higher cortisol levels were found in those with poorer cognitive function. Finally, the relationship between brain changes, (such as reduction in brain volume, termed "brain atrophy") seen on a brain scan taken at the time of admission (a Computed Tomography, or CT scan) and their relationship to who went on to develop delirium was investigated.

A study, involving 95 participants aged over 60 who had been admitted to hospital following a stroke, was conducted. Participants, or if they were not capable, their next of kin, gave informed consent to take part in the study. Brain CT scans, taken at the time of admission as part of clinical care, were assessed for changes, including reduction in brain volume, blood, areas of stroke damage and changes to the white matter of the brain. Participants were assessed on alternate days in the week after their stroke and at regular intervals thereafter, to see if they had developed delirium. Of the 95 participants, 26 (27%) developed delirium at some point during the study. A small sample of saliva was collected in the morning and afternoon on each assessment day, and levels of cortisol in the saliva were subsequently measured. Participants underwent brief testing of their memory and thinking at the time of recruitment, and an informant completed a questionnaire about the participant's memory in the period prior to their stroke. Participants were visited at 1 month, 4 months and 12 months after their stroke and were tested for delirium, underwent more detailed memory testing and had saliva samples collected to test for cortisol levels.

The study found that those who developed delirium did have slightly higher levels of cortisol than those without delirium, but once factors such as age and how severe the stroke was were accounted for, there was no difference in cortisol levels between the two groups. Those who had delirium were more likely to have cognitive impairment at follow-up, but taking the cohort as a whole, there was an improvement in cognitive function between the time of recruitment to the study and the 1 year follow-up, which is probably due to recovery from delirium and recovery from the acute effects of the stroke. Those who developed delirium were more likely to have volume loss of their brain, detected by CT scanning, but there were no differences between those with and without delirium when the other brain changes previously described were looked at. The studies have improved our understanding of the possible underlying mechanisms of delirium after stroke, and have improved our understanding of the natural history and characteristics of delirium after stroke. Finally, several areas for further research have been highlighted by these studies.

Abbreviations

ACE-R	Addenbrook's Cognitive Examination-Revised
ACH	Acetylcholine
ACS	Acute Coronary Syndrome
ACTH	Adrenocorticotropic Hormone
ADL	Activities of Daily Living
APACHE	Acute Physiology and Chronic Health Evaluation System
ARAS	Ascending Reticular Activating System
AUC	Area Under the Curve
BBB	Blood Brain Barrier
BI	Barthel Index
CAA	Cerebral Amyloid Angiopathy
CABG	Coronary Artery Bypass Graft
CAM-ICU	Confusion Assessment Method-Intensive Care Unit
CBG	Corticosteroid Binding Globulin
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSST	Digit Symbol Substitution Test
ELISA	Enzyme-Linked Immunosorbent Assays
GOS	Glasgow Outcome Scale
GP	General Practitioner
HPA	Hypothalamic-Pituitary-Adrenal
ICU	Intensive Care Unit
IL	Interleukin
IQ	Intelligence Quotient
IQCODE	Informant Questionnaire on Cognitive Decline in the Elderly
IQR	Interquartile Range
KI	Katz Index
LACS	Lacunar Stroke
MCA	Middle Cerebral Artery

MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
MRI	Magnetic Resonance Imaging
MRS	Modified Rankin Scale
NART	National Adult Reading Test
NHS	National Health Service
NIHSS	National Institutes for Health Stroke Scale
OCSP	Oxford Community Stroke Project
OSLA	Observational Scale of Level of Alertness
PACS	Partial Anterior Circulation Stroke
PACS	Patient Archiving and Communication System
POCS	Posterior Circulation Stroke
RASS	Richmond Agitation and Sedation Scale
SAH	Subarachnoid Haemorrhage
SIGN	Scottish Intercollegiate Guidelines Network
SPECT	Single Proton Emission Computed Tomography
SPSS	Statistical Package for the Social Sciences
STROBE	Strengthening Reporting of Observational Studies in Epidemiology
SSS	Scandinavian Stroke Scale
TACS	Total Anterior Circulation Stroke
WMLs	White Matter Lesions
UK	United Kingdom
11βHSD	11β Hydroxysteroid Dehydrogenase

Chapter 1: General Introduction

1.1 Stroke

In this section stroke will be defined and its epidemiology and pathophysiology will be described. The diagnosis of stroke and subsequent classification of stroke types and stroke severity will also be discussed.

1.1.1 Stroke definition

A stroke is defined as rapidly developing clinical signs of focal (at times global) disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin (Hatano, 1976).

1.1.2 Stroke epidemiology

Approximately 152 000 strokes occur in the United Kingdom (UK) annually, and the incidence of stroke is between 115-150 per 100 000 of the population (The Stroke Association, 2015). Over the last 15 years both the incidence of stroke and the overall mortality from stroke has fallen consistently, which has been attributed to better control of risk factors both before and after incident stroke, and also improved treatments for acute stroke (e.g. thrombolysis) (Lee et al., 2011b). Age is the most important risk factor, with the risk doubling with every decade after the age of 55, and 1 in 5 women and 1 in 6 men having had a stroke by the age of 75 (The Stroke Association, 2015). Other major risk factors include social deprivation, race (those of black and South Asian origin having a higher risk than white Europeans), hypertension, smoking cigarettes, hypercholesterolaemia, diabetes and atrial fibrillation. There are currently 1.2 million stroke survivors living in the UK, many of whom have been left with significant physical disabilities, fatigue and cognitive impairment (The Stroke Association, 2015). Minimising cognitive changes after stroke has recently been identified as the top priority for stroke survivors, their carers and clinicians (Pollock et al., 2014). Finally, stroke confers significant mortality, with 1 in 8 strokes being fatal within the first 30 days, and 1 in 4 strokes being fatal within the first year (The Stroke Association, 2015).

1.1.3 Stroke pathophysiology

The main pathological stroke types are ischaemic, primary intracerebral haemorrhage and subarachnoid haemorrhage, with ischaemic strokes accounting for 80% of all strokes in the UK (The Stroke Association, 2015). Ischaemic stroke may be divided into subtypes base on pathophysiology, using the TOAST classification system (Adams et al., 1993). This includes five categories: 1. Large-artery atherosclerosis, 2. Cardioembolism. 3. Small-vessel occlusion 4. Stroke of other determined aetiology (for example vasculopathies or haematological conditions) and 5. Stroke of undetermined aetiology. The net result of an ischaemic stroke, regardless of the aetiology, is ischaemia, and if reperfusion does not occur, infarction of brain tissue in the affected territories.

Haemorrhagic strokes are caused by blood vessel rupture, which leads to compression of brain tissue from the expanding haematoma. Haemorrhage usually occurs as a result of hypertensive vascular disease, arteriovenous malformation rupture or aneurysm rupture, although there are several other causes such as cerebral amyloid angiopathy, vascular tumours and infection (Weatherall, 1996). Cerebral amyloid angiopathy (CAA) is a frequent pathological finding in the elderly human brain, and is an accumulation of beta-amyloid in the walls of cortical blood vessels (Reijmer, 2015). Whilst CAA is generally thought to be a cause of haemorrhagic stroke, it is also known to be a risk factor for vascular cognitive impairment (Greenberg et al., 2004).

1.1.4 Stroke diagnosis

The diagnosis of stroke is a clinical one, relying on the history of an acute neurological event, along with clinical signs of neurological deficit, and with no apparent cause other than a vascular event. Neuroimaging (usually Computed Tomography, CT) is essential in distinguishing ischaemic from haemorrhagic stroke, and for excluding other underlying pathology which may mimic a stroke, such as a brain tumour. However, it is important to note that signs of ischaemia are often absent from a CT brain scan in the acute setting, particularly when scans are performed very early after symptom onset, but this does not preclude the diagnosis of stroke (providing no other pathology which may mimic a stroke is seen on neuroimaging) in the context of a salient history and examination. Neuroimaging in acute stroke will be discussed in more detail in section 1.5.

It is useful to categorize (or classify) the type of stroke a patient has had in clinical practice, and also for clinical research, in order that epidemiological studies can be performed to gain information about specific types of stroke, such as prognosis, and also in order that new treatments can be targeted and tested on patients who have had specific types of stroke.

1.1.5 Stroke classification

One conventional system for classifying stroke type used in clinical practice in the UK is the Oxford Community Stroke Project (OCSP) classification system (Bamford et al., 1991). This classification system was developed to create subgroups with patterns of characteristics which would be useful in both clinical practice and also in clinical research, but which do not require a detailed assessment of the pathophysiological mechanism underlying the particular stroke in order to allow classification. The OCSP classification system was chosen for this study, as it is widely used on stroke units in Scotland and also has been widely used in clinical research into delirium after stroke, thus allowing comparison between this study and those preceding it. The four subgroups are outlined in Table 1.1 in a simplified form. These subgroups broadly correspond to anatomical territories in terms of affected vasculature, however it is important to remember that the classification is based purely on clinical findings, and this does not always exactly match neuroimaging findings. In general, a Total Anterior Circulation Stroke (TACS) is a stroke affecting the territories supplied by the deep and superficial territories of the Middle Cerebral Artery (MCA), a Partial Anterior Circulation Stroke (PACS) affects the territories supplied by either the upper or the lower division of the MCA as well as individual branch occlusions and a Lacunar Stroke (LACS) usually relates to a lacune in the pons or basal ganglia. Posterior Circulation Strokes (POCS) are more difficult to categorise in terms of vasculature because of variable vascular anatomy and extensive collateral channels, consequently posterior strokes refer to those affecting the brainstem, cerebellum or occipital lobes (Bamford et al., 1991).

This classification allows predictions to be made about prognosis following stroke. A TACS has the poorest prognosis, with 60% dead at 1 year, 36% dependant and 4% independent. The prognosis for all four of the stroke subtypes is summarised in table 1.1(Bamford et al., 1991).

Table 1.1 Clinical features and prognosis for stroke by OCSP classification type

Stroke Subtype	**Clinical Features**	**Prognosis at 1 year (%)**
Total Anterior Circulation Stroke (TACS)	New higher cerebral dysfunction* and Homonymous hemianopia and Ipsilateral motor and/or sensory deficit	Dead 60 Dependant 36 Independent 4
Partial Anterior Circulation Stroke (PACS)	Higher cerebral dysfunction alone or 2 out of 3 of features of TACS or Restricted motor sensory deficit	Dead 16 Dependant 29 Independent 55
Lacunar Stroke (LACS)	Pure motor stroke or Pure sensory stroke or Sensori-motor stroke or Ataxic hemiparesis	Dead 11 Dependant 28 Independent 60
Posterior (POCS)	Ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit Bilateral motor and/or sensory deficit Disorder of conjugate eye movement Cerebellar dysfunction Isolated homonymous hemianopia	Dead 19 Dependant 19 Independent 62

*Such as dysphasia, dyscalculia or visuospatial disorder.

1.1.6 Stroke severity

There are several scales used to measure stroke severity, both for research and clinical purposes. Examples include the Scandinavian Stroke Scale (SSS) (Scandinavian Stroke Study Group, 1985), the National Institutes of Health Stroke Scale (NIHSS) (Brott et al., 1989) and the Canadian Neurological Scale (Cote et al., 1986). The NIHSS was chosen for this study as it is already routinely used in clinical practice on the Acute Stroke Unit in Edinburgh, it has been used in many previous stroke studies, and is relatively simple to score (with brief training). It is known to correlate with infarct size and long-term prognosis and predict discharge disposition (Siegler and Martin-Schild, 2015). The maximum score on this scale is 42 (with a higher score indicating a more severe stroke). A score of 1-4 is conventionally accepted to represent a minor stroke, 5-15 a moderate stroke, 16-20 a moderate to severe stroke and 21-42 a severe stroke (Brott et al., 1989). There are, however, some limitations to this method of rating stroke severity. For example a posterior circulation stroke resulting in significant vertigo may be rated as a minor stroke using this scale, and yet have a very significant effect on functional status and quality of life.

1.1.7 Summary

In summary, stroke is common and is associated with several adverse outcomes including significant physical disability, fatigue and cognitive impairment. Stroke has several pathophysiological sub-types, some of which may co-exist and diagnosis of a stroke is made based on clinical findings, although neuroimaging may help to confirm the diagnosis and distinguish haemorrhagic from ischaemic stroke. Finally stroke may be classified according to the clinical features found, using the OCSP system and also according to the severity as rated by a validated scale, such as the NIHSS. Both the OCSP classification and NIHSS score are associated with stroke outcome.

1.2 Delirium

In this section delirium will be defined and its core feature of inattention will be explored. The epidemiology and pathophysiology of delirium will then be described, and the diagnosis of delirium will then be discussed. Finally, delirium in the context of acute stroke will be discussed.

1.2.1 Delirium definition

Delirium, previously often commonly referred to as an "acute confusional state," is an acute and severe neuropsychiatric condition, characterised by its core feature of inattention. Inattention refers to disturbed consciousness and a reduced ability to focus, shift or sustain attention (American Psychiatric Association, 2000). Other cognitive features are also commonly seen including altered alertness, mood disturbance, disturbed sleep-wake cycle, and psychotic features such as delusions and hallucinations (Inouye et al., 2014).

1.2.2 Attention

As inattention is the core feature of delirium, I will discuss attention in general terms here. Attention is a complex part of cognition. During wakefulness, we are constantly encountering a multitude of external stimuli from the environment via all of our sensory organs, as well as being attentive to internal stimuli in the form of constant thoughts, ideas and memories. Despite these stimuli, we are able to perform a number of goal driven tasks, such as driving a car or solving complex problems, meaning that attentional processes allow us to focus on specific stimuli whilst holding other stimuli at bay, at least temporarily. Attention has been classified in various ways in the literature. Hodges (Hodges, 2007) provides a useful 4 component classification system outlined below:

1. Arousal. Describes the general state of wakefulness and responsivity.
2. Sustained attention. The capacity to maintain attentional activity over prolonged periods of time.
3. Divided attention. The ability to respond to more than one task at once
4. Selective attention. The ability to highlight or focus on one stimulus whilst suppressing awareness of competing stimuli.

The maintenance of attention depends on the interaction of two neural systems:

1. The ascending reticular activating system (ARAS)
2. Cortical regulation, involving limbic, parietal and prefrontal cortical regions.

The ARAS is said to provide 'bottom up' modulation of cortical regions and the cortex is said to provide 'top down' regulation via the thalamus (Hodges, 2007). In addition to these two systems of regulation, there are local processes, which operate in specialized domain specific regions, and modulate responsivity to sounds, tactile stimuli, faces, motion, words and memories.

Functional imaging studies have provided useful information as to the specific areas of the cortex involved in attention. For example, the parietal cortex has been found to be active during tasks requiring sustained and selected attention, and the prefrontal cortex is active in tasks requiring divided attention. Disorders of attention can, therefore, arise from a wide range of pathological processes involving any of the structures and pathways previously described. This may be in the form of structural damage, or be due to pharmacological agents or metabolic disorders (Hodges, 2007).

1.2.3 Delirium epidemiology

Delirium affects approximately 12 % of all hospitalised patients (MacLullich, 2013), and around 25% of those hospitalised following an acute stroke (Shi et al., 2012). In surgical units delirium is even more common, with an incidence of more than 40% reported after hip fracture (Freter et al., 2015) and around 28% after cardiac surgery (although rates varied widely depending on the type of surgery participants underwent) (Tse et al., 2015). Delirium is also commonly seen in Intensive Care Unit (ICU) patients, with an incidence of up to 80% reported in the literature (Girard et al., 2008). The differences in incidence rates between the various clinical settings are likely due to the variations in the patient populations found in each setting (for example those in an elective orthopaedic ward are likely to be younger and fitter than those on a Medicine of the Elderly ward), as well as the differences in the pathophysiological insult conferred by the condition for which the patient is hospitalised, and the variability in the underlying vulnerability conferred by comorbid disease. Incident rates for each individual setting vary between studies and this is likely due to a number of

methodological factors, including the country in which the study is conducted, method and frequency of assessment for delirium and the demographics of the study population.

Risk factors for delirium vary depending upon the clinical setting, although there are some common themes throughout. A recent systematic review of delirium incidence and risk factors for older adults in acute medical units found that dementia, illness severity, catheterisation, low albumin, visual impairment and length of stay were all associated with the development of delirium (Ahmed et al., 2014). Following hip fracture, prior cognitive impairment has been shown to be the strongest risk factor for developing delirium (Freter et al., 2015) and in the ICU setting age, dementia, illness severity and emergency surgery or trauma prior to admission have been shown to be associated with delirium (Zaal et al., 2015). Risk factors associated with delirium after stroke are discussed in detail in section 1.2.6.

1.2.4 Delirium pathophysiology

The pathophysiology of delirium remains incompletely understood. Several possible mechanisms have been postulated, which for clarity have been divided into seven main categories below (Maldonado, 2013). These mechanisms are mainly complimentary rather than competing, and it is probable that some, if not all of these mechanisms play a part in the pathogenesis of delirium. It is not known whether delirium after stroke is caused by the same pathophysiological mechanisms as delirium in other conditions (such as during the time of an infection), but as the features of delirium are uniform, as defined by Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM IV), irrespective of the precipitant to delirium, it is assumed that one or more of these mechanisms are as likely to be as pertinent to post-stroke delirium as they are to all other forms of delirium. One exception to this may be thalamic strokes, which may specifically affect attention (by the mechanisms described in the "attention" section), however these are relatively rare (Saez de Ocariz et al., 1996).

Neurotransmitters

Several neurotransmitters have been investigated with respect to their putative role in delirium pathophysiology. The most commonly described changes seen in delirium are a reduction in acetylcholine (ACh) (Flacker, 1998, Saez de Ocariz et al., 1996), and an increase in dopamine (Maldonado, 2013), in both blood and cerebrospinal fluid (CSF) (Hall, 2011). Acetylcholine is required for selective attention activities, and low levels of Ach have been

found in serum in those with delirium (Flacker, 1998). Furthermore, high levels of acetylcholinesterase, the main enzyme involved in the metabolism of Ach, have been found in CSF in those with delirium (Hall, 2011). Finally, medications with anticholinergic activity have been found to frequently precipitate delirium (Tune LE, 1999). High levels of dopamine metabolites have also been found in those with delirium, and it is hypothesised that dopamine may at least in part, be responsible for some of the behavioural changes commonly described, as dopamine has a role in attention, memory and perception (Trzepacz, 2000). Indeed, evidence of increased dopaminergic signalling has been found in the CSF of delirium patients with psychotic features (Ramirez-Bermudez, 2008).

Other neurotransmitters have also been implicated, for example, Serotonin is a monoamine neurotransmitter which has a role in cognition, mood and wakefulness, and it has been hypothesised that increased serotonergic activity can lead to delirium (Flacker, 1999). This hypothesis is supported by evidence of elevated 5-hydroxyindoleacetic acid (a metabolite of serotonin) in the CSF of those with delirium (Koponen, 1994) Finally, GABA is thought to be the main inhibitory neurotransmitter in the human CNS and GABA has been found to be elevated in some types of delirium and reduced in others (Maldonado, 2013).

Inflammation

The inflammation hypothesis is based on the premise that an acute stimulus, such as an infection, causes a cascade of pro-inflammatory cytokines, which cause neuronal and synaptic dysfunction. Many inflammatory cytokines, such as CRP, Interleukin- 6 (IL-6), IL-8 and Tumour Necrosis Factor have been found to be elevated in both blood and CSF in delirium when compared with controls, even after controlling for factors such as infection (de Rooij, 2007, Hall, 2011). The mechanism by which a minor infection, such as a urinary tract infection, without apparent systemic upset is able to precipitate delirium remains unclear.

Neuronal Ageing

Several age-related cerebral changes are hypothesised to contribute to the increased risk of delirium with age. These include neurone loss, reduced blood flow to the brain, and reduced vascular density in the brain. Pathological processes seen more commonly in ageing, such as those associated with Alzheimer's dementia, probably also increase the vulnerability of the brain to delirium, with CNS cells being chronically activated to produce an exaggerated inflammatory response to immune challenges (Maldonado, 2013).

Oxidative Stress

An acute insult such as infection may lead to tissue hypoperfusion, hypoxia and subsequent tissue damage. This in turn results in increased energy expenditure and reduced cerebral oxidative metabolism. The longer-term result of this may be damage to cerebral tissue and consequent longer-term cognitive decline (Maldonado, 2013).

Loss of Circadian Rhythm

Disruption of the normal twenty-four hour circadian rhythm may lead to disturbances in sleep architecture. Sleep deprivation has long been associated with delirium (Lipowski, 1987), and it has been shown that sleep debt can lead to poorer attention and reduced critical thinking. Whether loss of the normal circadian rhythm is a cause of delirium per se, or whether it simply aggravates or perpetuates delirium is not clear. Melatonin, with its sleep-wake cycle regulatory effects, actions as a free radical scavenger, as well as its anti-inflammatory properties and actions to reduce the affinity of glucocorticoid receptors, has been hypothesised as a potential treatment or preventative measure for delirium. A recent large randomised trial of melatonin after hip fracture did not shown any benefit in prevention of delirium (de Jonghe, 2014), however a recent systematic review and meta-analysis of all trials of melatonin (incorporating 4 randomised controlled trials, with a total n of 669) for delirium prevention found that there was a reduction in delirium incidence in patients admitted to medical wards, but not in those admitted to surgical wards, in the treatment groups (Chen et al., 2015). Further larger studies are across a range of hospital settings are now required to unpick the relationship between melatonin supplementation and delirium prevention.

Network Disconnectivity

This hypothesis is based on the premise that factors such as drugs, inflammation or infection affect neurotransmitter pathways and may cause them to fail or partially fail. Furthermore, the failure of any one pathway because of an acute insult will also affect the other neurotransmitter pathways, as they are intrinsically linked. Those subjects who do not have robust pathway (or “network”) connectivity due to ageing or cognitive impairment or reduced inhibitory tone (due to poor sleep, infection etc.) will hence be more vulnerable to delirium (Maldonado, 2013).

Aberrant Stress Response

The role of the stress response in delirium pathophysiology is the main focus of this thesis, and as such a detailed description of the hypothesis is given in sections 1.4.4 and 5.1. In brief, it is hypothesised that an acute physiological stressor (such as infection) triggers and then sustains high circulating levels of cortisol, which has physiological benefits in the short term including mobilisation of glucose from the liver and adipose tissue and suppression of the inflammatory responses. However, if the stress response is not suppressed in the normal way by a negative feedback mechanism, and circulating cortisol levels remain high, this may have deleterious effects in the brain, particularly in the hippocampus, which lead to delirium (MacLullich et al., 2008).

1.2.5 Delirium Diagnosis

The current gold standard for the diagnosis of delirium is based on the criteria set out in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), which are reproduced in table 1.2. Delirium can be divided into two main subtypes; hyperactive delirium, when inattention manifests itself as over activity and hypervigilance, and hypoactive delirium, when drowsiness and reduced frequency of movements are commonly seen (Inouye et al., 2014) In practice, fluctuation between these two states in an individual patient may also be seen.

Table 1.2 Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for the diagnosis of delirium

Criteria	Description
1	Disturbance of consciousness with reduced ability to focus, sustain or shift attention
2	A change in cognition or the development of a perceptual disturbance that is not better accounted for by a pre-existing, established or evolving dementia
3	The disturbance develops over a short period of time (usually hours to days) and tends to fluctuate during the course of the day
4	There is evidence from the history, physical examination or laboratory finding that the disturbance is caused by the direct physiological consequences of a general medical condition

The diagnosis of delirium is made using DSM-IV criteria as previously stated. Several screening tests, based around these diagnostic criteria, have been developed for use in a clinical setting to assess the features of delirium. The screening tests selected for this study (the Confusion Assessment Method for the Intensive Care Unit, the Delirium Rating Scale-Revised-98, Digit Span Forwards and Backwards and the Edinburgh Delirium Test Box Mark 2) which will be discussed in more detail in the methods chapter (section 4.4.5), were specifically chosen as they can be used on participants who are unable to verbalise (for example in those who are aphasic after a stroke).

1.2.6 Post-stroke delirium

According to a recent systematic review (Shi et al., 2012), approximately 25% of patients on the acute stroke unit will have delirium at some point during their hospital stay. The incidence of delirium found in the studies included in this review ranged from 10% to 48%, however this broad range is probably accounted for by methodological differences in the studies (frequency and method of delirium assessment), as well as changes in the way stroke care is provided (such as development of dedicated stroke units) (Shi et al., 2012). This is important, because delirium after stroke, as well as delirium associated with other illnesses

such as sepsis, is associated with multiple adverse outcomes, including longer-term cognitive impairment, longer length of stay, higher mortality and higher risk of institutionalisation (Sheng et al., 2006, Witlox et al., 2010, McManus et al., 2007, MacLullich et al., 2009). However, the mechanisms of post-stroke delirium remain poorly understood. It is recognised that, as in the non-stroke population, risk factors for delirium after stroke include infection and prior cognitive impairment, as well as factors specific to stroke such as increasing stroke severity (Oldenbeuving, 2013). Anterior circulation strokes are also associated with delirium (Oldenbeuving, 2011), but conflicting results have been found as to whether right or left hemisphere strokes confer the greater risk (Oldenbeuving et al., 2014, Henon et al., 1999).

Stroke as a cause or precipitant of delirium has been rather neglected from a research point of view, perhaps because it has been seen as a direct brain insult, which may at times be difficult to distinguish from delirium. It is recognised that stroke, particularly stroke affecting the thalamus, can cause attentional deficits and reduced conscious level (Kraft, 2015) . However, it is important to recognise that this is a distinct entity from delirium, with its fluctuating course and attentional and cognitive deficits which occur acutely and are a change from the patient's new baseline following stroke. This is an important point- this distinction is crucial if we are to recognise delirium in the stroke population and understand its pathophysiology in more detail. It is, of course, possible that those with a large thalamic stroke (or indeed a large TACS) may develop delirium around the time of stroke onset, and this would be difficult to distinguish from the stroke itself, but as previously discussed the incidence of thalamic stroke is low overall, and a pragmatic approach of looking for new changes following stroke and assessing all of the features of delirium in a detailed way ensures that the incidence is not over-estimated.

1.2.7 Summary

In summary, delirium is a common finding in patients post-stroke in hospital, and is associated with adverse outcomes. Delirium has two motor subtypes, hypoactive and hyperactive (and the two may co-exist, in that there may be rapid fluctuation between them). The diagnosis of delirium is based on clinical findings using the framework of the DSM-IV criteria, along with other screening tools to assess features in more detail. The pathophysiology of post-stroke delirium is incompletely understood, and several possible mechanisms have been investigated. One plausible pathophysiological mechanism is that of

the “aberrant stress response” which precipitates and then maintains delirium, and it is this putative mechanism which will be investigated in this thesis.

1.3 Cognitive impairment after stroke

One of the adverse outcomes associated with post-stroke delirium is cognitive impairment (van Rijsbergen et al., 2011). Indeed those who have an episode of delirium in the acute phase post-stroke, have between a five and seven fold increased risk of having dementia when assessed two years later (when pre-morbid cognitive impairment is controlled for using the IQCODE)(van Rijsbergen, 2011). Long-term cognitive impairment after stroke and its association with delirium in the acute phase after stroke will be explored in this thesis, and therefore cognitive impairment after stroke will be described in general terms in the following section.

1.3.1 Cognitive impairment definition

Cognitive impairment, as defined by the DSM-5 criteria (American Psychiatric Association, 2013) , may be divided into minor (sometimes called mild) or major cognitive impairment (often referred to as dementia -for the purposes of this thesis the two terms will be used interchangeably).

A minor cognitive impairment is defined by the following:

1. Evidence of modest cognitive decline from a previous level of performance and a decline in neurocognitive performance in the range of one or two standard deviations below appropriate norms on formal testing.
2. The cognitive deficits are insufficient to interfere with independence, but greater effort, compensatory strategies or accommodation may be needed to maintain independence.
3. The cognitive deficits do not occur exclusively in the context of delirium
4. The cognitive deficits are not primarily attributable to another mental disorder (such as depression)

A major cognitive impairment is defined by the following:

1. Evidence of a substantial cognitive decline in one or more domains and a decline in neurocognitive performance in the range of two or more standard deviations below appropriate norms on formal testing.
2. The cognitive deficits are sufficient to interfere with independence (requires assistance with activities of daily living).
3. The cognitive deficits do not occur exclusively in the context of delirium

4. The cognitive deficits are not primarily attributable to another mental disorder (such as depression)

1.3.2 Cognitive impairment epidemiology

Stroke and cognitive impairment are interrelated because they share similar risk factors and after stroke around 10% of patients without pre-stroke cognitive problems will develop cognitive impairment (Pendlebury, 2009).The overall incidence of post-stroke cognitive impairment at 6 months is approximately 20%, rising to approximately 22 % at 1 year (Pendlebury, 2012b) (the overall incidence of dementia in the over 65's in the UK is around 3%) (Prince, 2014). Cognitive impairment is an important long-term consequence of stroke, and is strongly associated with significant functional deficits (Mok et al., 2004) and new institutionalisation (Pasquini et al., 2007).

1.3.3 Cognitive impairment pathophysiology

The mechanisms of cognitive impairment after stroke are incompletely understood. The stroke lesion causes damage to a focal area of brain, however the effects seen on cognition tend to be more global. It has been hypothesised that this may in part be due to the strategic location of the infarct, for example affecting the thalamus, which might result in a more global cognitive decline, and that both single and multiple lesions may interrupt intracerebral circuits, for example by disrupting white matter connectivity (Pendlebury, 2012a). It is likely that both large vessel disease (atherosclerosis and other arteriopathies) and small vessel pathology (such as arteriosclerosis, amyloid angiopathy and other intracerebral vasculopathies) play a part in the pathophysiology of vascular cognitive impairment. Large vessel disease probably contributes to white matter pathology and causes impaired cerebral blood flow (even in the absence of an infarct), although the clinical significance of this is uncertain (Iemolo et al., 2009). Small vessel disease leads to cerebral tissue damage, usually lacunar infarcts and white matter changes (Iemolo et al., 2009).

Pathological studies have demonstrated that cerebrovascular disease contributes to the majority of cases of late onset dementia (including those diagnosed in life with Alzheimer's

dementia) (Savva and Stephan, 2010). Indeed, for dementia of any cause, vascular lesions have been shown to have a higher attributable risk than amyloid-beta plaques and neurofibrillary tangles combined (Matthews et al., 2009). Cerebrovascular disease and neurodegenerative pathology (such as that found in Alzheimer's Disease) frequently co-exist in the elderly, and the presence of both pathologies lowers the threshold for dementia (Snowdon, 1997). It is not clear whether stroke disease unmasks previous subclinical degenerative pathology, or works synergistically to accelerate degeneration and development of a clinical cognitive impairment. It should also be noted that vascular changes such as hypoperfusion may underlie the pathological changes seen in Alzheimer's disease (such as amyloid-beta plaques) (Pendlebury, 2012a). Determining the relative contributions of vascular and degenerative pathology to cognitive decline post-stroke is difficult, partly because several of the features are overlapping (for example, atrophy of the temporal lobes). Glucocorticoid excess may also have a role to play in the pathophysiology of cognitive impairment, and this hypothesis is discussed in detail in section 1.4.3.

Those with brain atrophy are known to be at higher risk of developing a major cognitive impairment after stroke (Area Under Curve (AUC) = 0.71, 95% CI 0.64-0.77), and those with white matter lesions (WMLs) may also be at higher risk (AUC = 0.59 CI 0.59-0.65)(Saini et al., 2014)·(Pendlebury, 2012a). These two specific pathological changes are discussed in depth in the neuroimaging section of this chapter (section 1.5).

1.3.4 Diagnosis of cognitive impairment

The clinical diagnosis of a cognitive impairment after stroke relies on the history (ideally given by an informant, such as the closest relative) of the person's ability to live independently as well as formal testing of cognition using one of the published tests. The cognitive tests most commonly employed for this purpose in the hospital setting in the UK are the Mini-Mental State Examination (MMSE) (Folstein et al., 1975), The Addenbrook's Cognitive Examination-Revised (ACE-R) (Mioshi et al., 2006) and the Montreal Cognitive Assessment (MoCA) (Nasreddine, 2005). More detailed neuropsychological tests are also available, which can test specific domains in more detail. The MoCA was selected as the main multi-domain cognitive test for this study, because it takes only 10 minutes to administer (in contrast to the ACE-R which takes around 20 minutes), and has been shown to

pick up more cognitive deficits than the MMSE in patients with stroke, and in particular the MoCA is able to demonstrate deficits in executive function and attention, which the MMSE is not (Pendlebury, 2010). More detailed neuropsychological tests were also performed as part of the study during follow up visits and these are described in detail in the General Methods chapter (section 4.6). Previous cognitive ability is also relevant and crystallised intelligence (and hence pre-morbid intelligence in those with cognitive decline) was therefore measured using the National Adult Reading Test (NART Nelson, 1991), which is also described in more detail in section 4.6.1. Finally an informant history was taken about the participants ability to live independently, and at the time of recruitment an informant completed the Informant Questionnaire on Cognitive Decline in the Elderly IQCODE (Jorm, 1995a) to screen for undiagnosed pre-existing dementia (section 4.6.2).

1.3.5 Classification (including severity) of cognitive impairment after stroke

In the DSM-5 criteria, the term "dementia" has been replaced by the term "major neurocognitive impairment." However this change has not reached clinical practice nor research publications to date and for the purpose of this thesis the two will be used interchangeably. The distinction between mild and major cognitive impairment are outlined in the definition section (section 1.3.1). Those with either a mild or major cognitive impairment with clinical and/or radiological cerebrovascular disease may be diagnosed with a vascular cognitive impairment, however Alzheimer's dementia pathology and vascular pathology frequently co-exist and it may be difficult to determine which patients have a single pathological process and which do not. Roman and colleagues, as long ago as 1993, published the NINDS-AIREN criteria for diagnosis of vascular dementia, which have been used extensively in clinical research (Roman et al., 1993). These criteria state that probable vascular dementia requires the presence of dementia along with the presence of cerebrovascular disease (focal neurological signs and evidence of recent cerebrovascular disease on brain imaging and a link between the two). The link between the two requires either a temporal relationship (onset of cognitive impairment within 3 months of the cerebrovascular disease), or a "classical clinical course," meaning an abrupt onset and stepwise course. The diagnosis of probable vascular dementia can be made if brain imaging criteria are not met or if the temporal relationship between cerebrovascular disease and dementia is not clear (Roman et al., 1993). A minor vascular cognitive impairment must fit

the criteria outlined in the definition section and be attributable to a vascular cause, as outlined above.

1.3.6 Summary

In summary, cognitive impairment is common after stroke and is a major concern for stroke survivors and their carers. Vascular cognitive impairment can be sub-divided into minor and major categories. The pathophysiology of vascular cognitive impairment frequently co-exists with other pathology such as that seen in Alzheimer's dementia. The diagnosis is made based on informant history (and participant history if possible) along with clinical examination and cognitive testing. Delirium is associated with cognitive impairment after stroke, in that those who develop delirium are more likely to have developed cognitive impairment at 2 years post-stroke (van Rijsbergen et al., 2011). Conversely, those with pre-existing cognitive impairment have a higher risk of developing post-stroke delirium (Oldenbeuving, 2011).

1.4 Glucocorticoids

As this thesis will be examining the associations between delirium after stroke and cortisol, I will discuss glucocorticoids (cortisol in humans, corticosterone in rodents) in general and then in relation to stroke specifically in the following section. A systematic review of the literature in relation to cortisol and stroke follows the introduction section (chapter 2).

1.4.1 Physiology

Glucocorticoids are hormones produced by the adrenal cortex in response to physical or psychological stress. The release of these hormones is under the control of the hypothalamic-pituitary-adrenal axis (HPA axis), which has a negative feedback mechanism. A schematic diagram of the HPA axis is shown below (Figure 1.1). Cortisol is transported in the blood bound to corticosteroid-binding globulin, a high affinity transporter, which is saturable. Serum albumin acts as an additional transporter, but has low affinity for cortisol. Free cortisol can be detected in saliva, and salivary cortisol levels have been shown to correlate with serum levels in healthy elderly adults (Reid et al., 1992). Glucocorticoid receptors are found in almost every cell in the body. Cortisol inhibits glucose uptake by peripheral tissues, which is thought to be in order to spare glucose for the brain, and stimulates gluconeogenesis, proteolysis and lipolysis. It also inhibits inflammatory pathways (Weatherall, 1996).

The HPA pathway is entrained on the circadian cycle, producing fluctuations which are cyclical, occurring every 24 hours. More specifically, the HPA axis exhibits diurnal variation, with a characteristic peak of cortisol being produced in the early morning and a nadir occurring in the late afternoon. This corresponds to the daily activity-rest cycle, and alteration of activity patterns (for example working at night) results in a slow, but corresponding, re-phasing of the HPA axis (Herbert et al., 2006). The circadian secretion of adrenocorticotrophic hormone (ACTH) from the pituitary is controlled by the suprachiasmatic nuclei, via a self-sustaining transcriptional/translational feedback loop, and is synchronised by input from photoreceptive retinal ganglion cells that project directly into the suprachiasmatic nuclei (Herbert et al., 2006). In addition to these diurnal fluctuations in cortisol, several stressors (both internal and external), via effects on the HPA axis, may cause a rise in cortisol levels. For example, cortisol is released in response to physiological stress

and hypoglycaemia (Weatherall, 1996) and elevated cortisol levels have also associated with psychologically stressful life events (Herane Vives et al., 2015).

An exaggerated response to physiological and psychological stressors, leading to higher levels of circulating cortisol, has been shown to be associated with several diseases including Alzheimer's disease and depression (Lupien et al., 1999). A meta-analysis of studies comparing healthy older volunteers, with younger healthy volunteers found that the elderly had an exaggerated cortisol response to physiological, psychological or pharmacological challenge (Otte, 2005). The study also found that the effect of age on cortisol response was greater in women than men, and the authors hypothesise that this may be related to the decrease in oestrogen in post-menopausal women, in some way attenuating the cortisol response (males do not undergo such a dramatic change in sex hormone levels with ageing) (Otte, 2005). In a cross-sectional study of 745 elderly participants, Johar and colleagues found that frailty is associated with a blunted diurnal cortisol pattern (evidenced by lower morning and higher evening cortisol levels) (Johar et al., 2014). However, the number of participants who were frail in the study (as defined by the study protocol) was low, at only 25 (3.4%), and participants were required to be able to walk, in order that gait speed be measured, meaning that the frailest, with poor mobility, were excluded. The dampening of diurnal variation in cortisol and the loss of negative feedback, resulting in persistent activation of the HPA axis with stress, has been hypothesised to be caused by age-related adaptations of both the central nervous system and endocrine system (Ferrari and Magri, 2008). For example, the suprachiasmatic nuclei have been shown to shrink with ageing and age related impairment of the zona reticularis of the adrenal cortex has also been demonstrated (Ferrari and Magri, 2008).

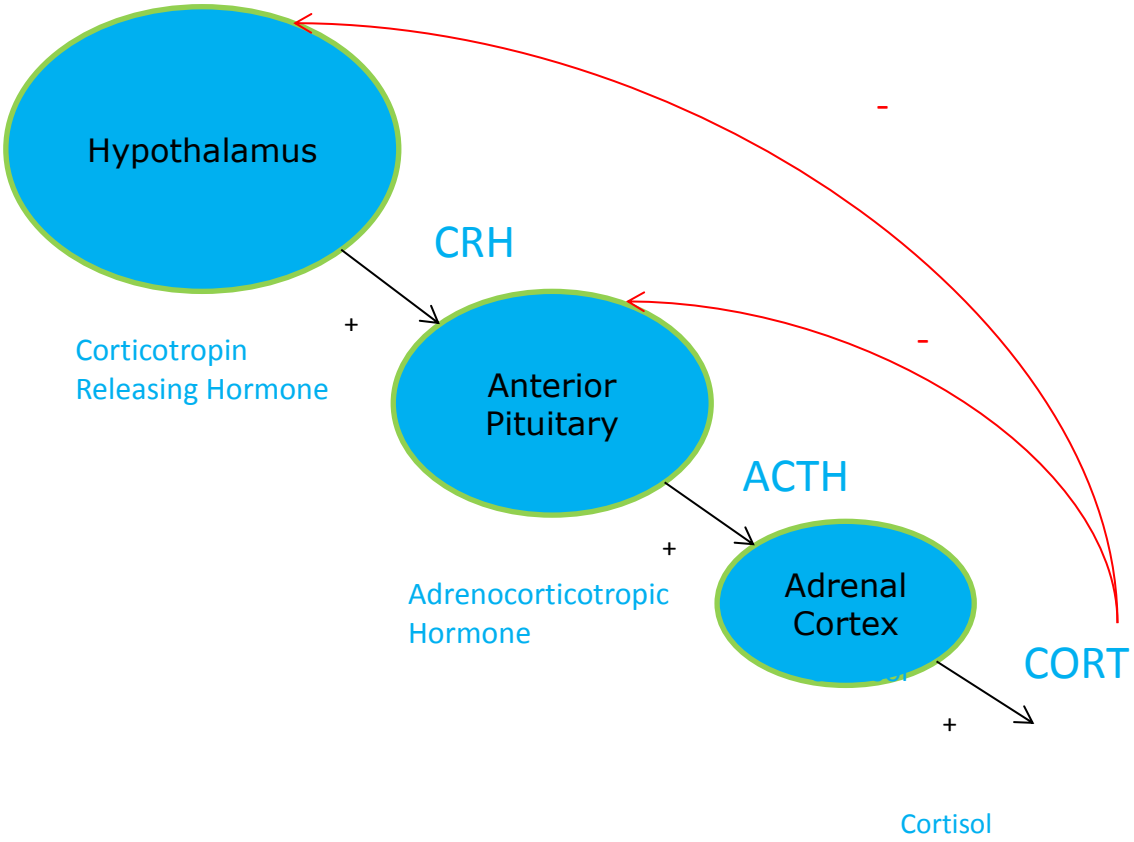


Figure 1.1 Schematic diagram of the hypothalamic-pituitary-adrenal axis

1.4.2 Glucocorticoids and stroke

Stroke is linked with an increase in the activity of the HPA axis, resulting in elevated circulating levels of cortisol (Fassbender et al., 1994, Feibel et al.,1977, Olsson et al., 1992). In general there is considerable individual variability in the degree and duration of cortisol elevation. Increased variability and a tendency towards dysregulation (resulting in abnormally prolonged HPA axis activation) is seen with ageing and especially in the presence of neurodegenerative disease (MacLullich et al., 2005). More prolonged HPA axis activation after a stroke may also occur for reasons specific to stroke. These reasons include cytokine release following neuronal injury, and that the stroke lesion itself may destroy HPA inhibitory areas of the brain in the frontal or medial temporal lobes (Mitchell, 1997). In order to explore the relationship between the HPA axis and stroke I have performed a systematic review of the published literature on this subject, the results of which are reported in chapter 2.

1.4.3 Glucocorticoids, the brain and cognition

The primary source of glucocorticoids in the brain is from the blood (Herbert et al., 2006). Glucocorticoids are primarily bound to corticosteroid-binding globulin (CBG) in blood, and CBG is the major limiting factor in diffusion across the blood brain barrier (BBB). Glucocorticoids themselves are relatively lipid soluble, and as such are able to diffuse across the BBB when unbound (Herbert et al., 2006). As CBG has limited binding capacity, any sudden rises in blood cortisol may lead to disproportionate rises in cerebrospinal fluid cortisol. P-glycoprotein transporters in the brain play a role in exporting cortisol across the BBB back into the blood, but are not thought to play a role in importing cortisol from the blood to the brain (Mason et al., 2010). Once cortisol enters the brain it is regulated by 11β-hydroxysteroid dehydrogenase activity (11β-HSD). This enzyme has two isoforms- 11β-HSD1 and 11 β-HSD2, which work in opposing directions, with 11β-HSD1 generating cortisol from its inactive 11-keto precursor (cortisone in humans), and 11 β-HSD2 inactivating cortisol (Mason et al., 2010). The 11β-HSD1 isoform is widespread in the human brain as well as in liver and adipose tissue, whereas the 11 β-HSD2 isoform is scarcely expressed in the human brain, being predominantly found in epithelial cells of the colon, kidney and salivary glands (Herbert et al., 2006).

Glucocorticoids have important effects on the brain, such as alteration of neuronal excitability throughout the diurnal cycle and modification of structural and neurochemical features of the brain following a stressor (Lupien et al., 1997). Corticosteroid hormones act via intracellular receptors that mediate slow genomic actions, however corticosteroids also have more rapid effects, which are thought to be mediated by actions on membranes and neurotransmitters (Lupien et al., 1997). There are two types of corticosteroid receptors in the human brain, Type 1 (or mineralocorticoid), which have a high affinity for cortisol and aldosterone, and Type 2 (or glucocorticoid) which have a lower affinity for aldosterone. When levels of circulating corticosteroids are low (such as after adrenalectomy), there is an increase in the number and also the affinity of the corticosteroid receptors (up-regulation), and conversely, when levels of corticosteroids are high (such as in Cushing's disease) there is a decrease in the number and affinity of the receptors (down-regulation). Corticosteroids have both immediate and delayed actions on neurones. The immediate action is to rapidly depress the firing activity of the neurones and the delayed action is to reduce the ability of neurones to respond to subsequent acute stimuli, and this effect can last for hours or even days (Lupien et al., 1997). Mineralocorticoid receptors are particularly prevalent in the limbic system, and

glucocorticoid receptors are widely expressed in most brain regions. The hippocampal formation, which has a key role in new learning and immediate memory, is known to contain the highest concentration of glucocorticoid binding sites in the entire brain (Starkman et al., 1992). Interestingly, prolonged exposure to high (non-basal) levels of corticosterone has been shown to be associated with hippocampal atrophy in rodents (Murray et al., 2008) and in humans with Cushing's disease (where cortisol levels are pathologically high), the hippocampal formation volume is negatively correlated with plasma cortisol levels and those with smaller hippocampal formation volumes have poorer verbal learning and verbal recall scores, even after correcting for prior cognitive ability (Starkman et al., 1992). Following successful treatment for Cushing's disease (leading to physiological cortisol levels being restored) the hippocampal formation volume has been found to increase by up to 10%. However, this study included only 22 participants, and although 18/22 showed an increase in hippocampal formation volume (with the remaining 4 showing no change) larger studies will be required to confirm this finding (Starkman et al., 1999). Declarative memory (voluntary or conscious recollection of previous information), in which the hippocampus plays a key role, is particularly affected by corticosteroids, adding weight to the hypothesis that the hippocampus is central to understanding the effects of corticosteroids on memory (Newcomer, 1994).

In rodent animal models and in humans, corticosteroids have been shown to have beneficial effects on memory at basal levels (Lupien et al., 2007). However, higher levels of corticosteroids have been found to have detrimental effects in a dose dependant fashion, and as such the relationship between corticosteroids and memory has been described as a U shaped curve (Lupien et al., 1997). There is a large literature describing the association between dysregulation of the HPA axis, resulting in elevated cortisol and chronic cognitive dysfunction and dementia (Herbert et al., 2006) (MacLullich et al., 2005). For example, in a cross-sectional study of 967 older community-dwelling adults, elevated cortisol levels were associated with poorer cognitive function (Lee et al., 2007), and in patients with Alzheimer's disease higher cortisol levels are associated with faster cognitive decline (Csernansky et al., 2006) . This is discussed in detail in section 7.1

1.4.4 Glucocorticoids and delirium

One of the fundamental hypotheses as to the pathophysiological mechanisms of delirium, that explains the clinical features of delirium and its association with physiological stress, is that it is triggered and then sustained by high circulating levels of cortisol (MacLullich et al., 2008). This hypothesis is supported by convergent evidence from basic science and clinical research. The phenomenon of "steroid psychosis," that is the development of significant changes in behavior and mood following the administration of corticosteroid medication, has been the subject of research and debate for many decades. There has been particular interest in how corticosteroids may regulate behavior, and how additional exogenous steroids may therefore interrupt this mechanism, resulting in the observed psychosis. Animal studies of the phenomenon include those conducted by Wolkowitz, who administered corticosteroid or placebo to rats and found that those given corticosteroids had significant increases in caudate homovanillic acid, a dopamine metabolite (Wolkowitz, 1994). Changes in behavior which correspond to dopamine activity, such as increased locomotion, were also observed (Wolkowitz, 1994). Interestingly, Lithium pre-treatment of the rats was found to prevent the occurrence of both the biochemical and behavioral effects of corticosteroids (Wolkowitz, 1994). The same group also performed a number of human studies, and found that administration of Dexamethasone to healthy volunteers resulted in elevated plasma homovanillic acid levels. A second study in which 12 healthy volunteers were given a 5 day course of placebo, followed by 5 days of oral Prednisolone (80mg) and then 7 days of placebo (using a double-blind methodology), found that Prednisolone was associated with diverse and fluctuating behavioral changes, including depression and anger, in 75% of participants. Both study groups underwent cognitive testing during the period of corticosteroid administration. Neither Dexamethasone nor Prednisolone were associated with altered attention per se, but were associated with errors of commission, which refers to the inability to filter out distractors during testing (Wolkowitz, 1994).

1.4.4.1 Clinical studies of glucocorticoids and delirium

There is mounting direct evidence from clinical studies linking glucocorticoids with delirium (MacLullich et al., 2008).

Peri-operative studies

Kudoh and co-workers (Kudoh, 2005) found that elderly patients (age range 70-90) who developed post-operative delirium had higher post-operative cortisol levels, although despite controlling for surgery duration, age and anaesthesia length, they did not control for post-operative illness severity. Kazmierski and colleagues investigated 113 participants undergoing coronary artery bypass grafting (CABG) surgery, and found that raised peri-operative plasma cortisol levels were associated with the development of delirium, although only 2 plasma levels were measured, one in the morning prior to surgery and one on the morning following surgery (Kazmierski et al., 2013). Mu and colleagues also investigated the relationship between plasma cortisol levels and delirium in a cohort of 243 participants undergoing elective CABG surgery. They also found an independent association between elevated cortisol and post-operative delirium, after controlling for other confounders such as age and illness severity (Mu et al., 2010). A meta-analysis of studies investigating the predisposing and precipitating factors associated with delirium after cardiac surgery (and which included the 2 studies discussed here) concluded that elevated cortisol is one of the most established precipitating factors of delirium after cardiac surgery (Lin et al., 2012). Bisschop et al investigated the associations between plasma cortisol and delirium in a cohort of 143 participants following hip fracture and found a trend towards higher cortisol levels in those who developed delirium, but this was not statistically significant in multivariate analysis (Bisschop et al., 2011). More recently Hall et al investigated a cohort of 104 participants with hip fracture, and found an association between morning cortisol levels and delirium after adjusting for age, illness severity, prior cognitive impairment and co-morbidities (Hall et al., 2015).

Medical and ICU studies

O'Keeffe and Devlin (O' Keeffe, 1994) studied the relationship between delirium and the dexamethasone suppression test in 16 elderly patients with lower respiratory tract infection. Seven of the nine patients with delirium, and only one of the seven non-delirious patients, were non-suppressors. Colkeson et al investigated the relationship between serum cortisol and delirium in 52 patients presenting with an acute coronary syndrome (ACS) and found an independent association with elevated cortisol and delirium, after controlling for age, sex and severity of the ACS, however dementia and co-morbid disease were not controlled for (Colkesen et al., 2013). A significant correlation between delirium severity (measured by the DRS) and cortisol levels was also demonstrated. In a critical care setting, Nguyen et al

(Nguyen et al., 2014) investigated the relationship between plasma cortisol and delirium in those with sepsis (n=128). Delirium (or brain dysfunction, as it was termed in this study) before sedation was defined as a positive CAM-ICU or a Glasgow Coma Scale of ≤13, and after withdrawal of sedation was defined as a positive CAM-ICU. Delirium was diagnosed in 84% of participants after sedation withdrawal (104/128). Cortisol was measured only in the morning rather than diurnally, and so no information about the diurnal cortisol variability is available from this study. The authors found an independent association between elevated cortisol and the development of delirium after controlling for confounders (Nguyen et al., 2014).

Stroke studies

There is some specific evidence directly linking HPA axis dysregulation with delirium in stroke patients. Two studies have reported that non-suppression to dexamethasone is associated with a higher risk of delirium (n = 99)(Gustafson Y, 1993, Olsson et al., 1992) and one study has reported higher cortisol levels in those with delirium (n = 88) (Marklund et al., 2004). These studies are discussed in more detail in section 5.1.

1.4.5 Summary

In summary, glucocorticoids have important functions throughout the body, including the brain, and play a central role in the stress response. Abnormally high levels of cortisol have been linked with cognitive impairment, and this may be due to its detrimental effects on the hippocampus. Delirium has been shown to be associated with higher cortisol levels in a variety of clinical scenarios, including after hip fracture and following surgery. Three small studies have found an association between cortisol after stroke and delirium, but all are limited by methodological weaknesses, which are discussed in more detail in section 5.1.

1.5 Neuroimaging

Neuroimaging is performed on nearly all stroke patients and neuroimaging analysis is an important area of investigation into the pathophysiology of delirium. As image analysis will form part of this thesis, I will discuss imaging modalities and potential findings in more detail below.

1.5.1 Imaging modality

The majority of stroke patients undergo brain imaging, usually with computed tomography (CT) around the time of admission (within 24 hours of stroke onset), as recommended in the current national Scottish Intercollegiate Guidelines Network (SIGN) guidelines (www.sign.ac.uk). Magnetic Resonance Imaging (MRI) is not routinely available for all stroke patients and is particularly problematic in this patient group, because of the time required to complete the scan and also because MRI can be distressing and noisy for patients to tolerate. Furthermore MRI may not reveal hyperacute haemorrhage correctly, and up to one fifth of patients are not able to undergo MRI scanning, either because they are too unwell or because they have an intracerebral or intraocular metallic foreign body or a pacemaker (Wardlaw, 2004). In the context of an acute stroke, CT brain scanning is used to detect acute areas of hypo or hyperattenuation which indicate an ischaemic or haemorrhagic stroke respectively. The scan will, however, detect other changes in the brain which may or may not be related to the acute stroke. For example changes to the white matter (in regions other than that of the acute stroke, often referred to as white matter lesions (WMLs)) are frequently seen in stroke patients (Leys, 1999) as is brain volume loss (often referred to as brain atrophy), which may be global or localised to specific regions. These two specific changes (The presence of WMLs and brain atrophy) are of particular interest as they have both (either in combination or individually) been associated with deterioration in cognitive ability (Leys, 1999, Ikram, 2008).

1.5.2 White Matter Lesions

White matter lesions (WMLs) are patchy or confluent periventricular and subcortical areas of lower density on CT scans (they can be seen as high signal intensity areas on MRI). The aetiology of WMLs remains obscure, but they are assumed to be of vascular origin (Kalaria, 2012). The pathogenesis of WMLs includes reactive gliosis, shrinkage of oligodendrocytes, ischaemic demyelination and transient oedema, which are thought to result from vascular insufficiency and a chronic hypoxic state (Kalaria, 2012). Shrinkage of oligodendrocytes seems to lead to eventual cell death with consequent loss of numbers of these cells in the white matter (Ihara et al., 2010). Mild ischaemic injury is known to activate myelin repair, however prolonged ischaemia is thought to damage oligodendrocyte precursor cells with a net result of unsuccessful remyelination (Simpson et al., 2007). WMLs are associated with cerebrovascular risk factors, particularly hypertension and advancing age (Salat et al., 2005). WMLs and stroke are closely associated, with WMLs being a risk factor for first ever and for recurrent stroke (Grueter and Schulz, 2012). This can probably, at least partially, be explained by their shared risk factors such as hypertension. WMLs have been found to be present in up to 40% of those with a diagnosis of Alzheimer's dementia and are also a common pathological change seen in vascular dementia (Leys, 1999). It is postulated that changes to the white matter is a mechanism of age related cognitive decline. One possible mechanism by which this may come about has been termed "cortical disconnection," a term first described by Geschwind in 1965(Geschwind, 2010) but which has gained credence following the results of several functional neuroimaging studies which have confirmed loss of white matter connectivity associated with advancing age (D'Esposito, 1999, O'Sullivan et al., 2001). The cortical disconnection theory is based on the concept that loss of white matter fibres leads to the loss of functional integration of neurocognitive networks, which in turn leads to the clinical picture of cognitive impairment (O'Sullivan et al., 2001). Finally, WMLs are associated with brain atrophy, even when shared risk factors are controlled for (Appleman, 2010).

1.5.3 Brain Atrophy

Global (or whole brain) atrophy has been related to increased risk of dementia in several neuroimaging studies (Erten-Lyons et al., 2006). Global brain atrophy, characterised by narrowing of the gyri, widening of the sulci and enlargement of the ventricles, is a common finding on CT or MRI in the elderly (Appleman, 2010). The extent and rate of total brain atrophy and ventricular enlargement are associated with development of dementia, independent of hippocampal atrophy (Appleman, 2010). However, it is important to note that there is a life-long association between whole brain size and general cognitive ability, and so when investigating the contribution of global or regional brain atrophy to overall cognitive status, it is important that prior maximum brain size (estimated using intracranial area) and peak cognitive ability are taken account of (Shenkin, 2009).

Risk factors for brain atrophy are similar to those associated with WMLs, namely age and hypertension, but also diabetes and cigarette smoking, reflecting once again the presumed vascular origin of the pathology (Appleman, 2010).

Medial temporal lobe atrophy, and in particular hippocampal atrophy, is closely related to memory impairment and is a predictor of Alzheimer's dementia (hippocampal atrophy corrected for intracranial volume, to account for prior brain volume) (Ikram et al., 2010), even in those who are currently not symptomatic. Hippocampal neurones are extremely vulnerable to hypoxia which may be caused by systemic vascular disease. However, it is not clear whether hypoxia is the sole underlying mechanism for development of cerebral atrophy per se, or whether other mechanisms may be involved (Kalaria, 2012). It is now clear, from neuroimaging studies and from The Nun Study (Snowdon, 1997) that medial temporal lobe atrophy is not restricted to Alzheimer's disease, and also that there is considerable pathological overlap between vascular cognitive impairment and Alzheimer's disease.

1.5.4 White Matter Lesions, Brain Atrophy and Delirium

Currently, there are very few neuroimaging studies in delirium. A systematic review in 2008 identified twelve neuroimaging (CT, MRI, Single Proton Emission Computed Tomography (SPECT)) studies that had studied a total of 194 patients with delirium and 570 controls (only four of these studies included stroke patients)(Soiza et al., 2008). Though delirium appeared

to be more common in patients with white matter lesions, the studies were very small, and did not control for confounding factors. To my knowledge just two published studies have investigated neuroimaging features after stroke in relation to delirium risk. Henon and colleagues recruited 202 participants and found that those with delirium had greater degrees of cortical atrophy and white matter lesions ((WMLs), 120 had a computed tomography (CT) scan and 82 had a Magnetic Resonance Imaging (MRI) scan), but there was no correction for age.(Henon et al., 1999) Oldenbeuving and colleagues reported that in 484 stroke participants, brain atrophy on CT scan (13 brain regions rated and scored combined to give an overall brain atrophy score) was associated with an increased risk of delirium in the first week after stroke, but did not re-assess participants after the first week, unless they were diagnosed with delirium (Oldenbeuving, 2011).

1.5.5 Stroke Lesion and Delirium

Few studies have investigated the relationship between the stroke lesion (type, site and size) and delirium. Those that did reported conflicting results. For example, Gustafson and colleagues (Gustafson Y, 1993) studied 83 participants after stroke and found that delirium was associated with haemorrhagic stroke and left sided lesions, however Oldenbeuving and colleagues (Oldenbeuving, 2011) studied 484 participants and found an association between right hemisphere lesions and delirium. Henon and colleagues (Henon et al., 1999) examined the relationship between old infarcts seen on neuroimaging and silent infarcts and found no association between either of these features and delirium.

1.5.6 Summary

In summary, neuroimaging offers the potential to improve our understanding of the pathophysiological processes underpinning the development of delirium after stroke. Both acute (in the form of any visible acute stroke lesions) and chronic (in the form of any old stroke lesions, WMLs and brain atrophy) changes can be assessed from clinical CT brain scans. CT scanning has the advantage of being suitable for almost all stroke patients, ensuring that bias is not introduced by relying on MRI (which is most suitable for those with less severe strokes). Previous studies, presented in this section, support the need for further work in this area to elucidate whether white matter lesions, stroke lesions, and other factors such as

brain atrophy, seen on clinical CT imaging, are associated with the development of delirium. The findings from such studies may be useful, in conjunction with clinical measures, in aiding clinicians to predict who is particularly at risk of delirium. This may be especially useful for predicting those likely to develop hypoactive delirium which is may be under-recognised. Strategies to reduce the risk of delirium in the most vulnerable can then be considered and targeted appropriately.

1.6 Aims, objectives and hypotheses of this thesis

The main aim of this thesis is to investigate the role of hypothalamic-pituitary-adrenal (HPA) axis dysregulation (resulting in elevated cortisol levels) in delirium and long-term cognitive impairment after stroke.

My key objectives are to determine:

1. Whether delirium after stroke is associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis
2. Whether dysregulation of the HPA axis in the week after stroke predicts cognitive decline up to one year
3. Whether delirium after stroke is associated with the development of cognitive decline up to one year
4. Whether features detectable on baseline clinical Computed Tomography (CT) scans (e.g. global atrophy, white matter lesions) can predict the development of delirium following stroke.

My core hypotheses are:

1. Delirium after stroke is associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis as indexed by elevated morning and afternoon cortisol levels, and reduced morning: afternoon cortisol ratios.
2. Dysregulation of the HPA axis as indexed by elevated morning and afternoon cortisol levels, and reduced morning: afternoon cortisol ratios in the first week after stroke predicts cognitive decline at up to 12 months.
3. Delirium after stroke is associated with decrements in cognitive function over one year.
4. Baseline pathology detected on admission CT brain scans, namely global cerebral atrophy, white matter lesions and visible acute stroke lesions, predict the development of delirium after stroke.

Chapter 2: Glucocorticoids and stroke- a systematic review of the literature

The following systematic review has been published in The Journal of Neurology (Barugh et al., 2014)(see appendix 9 for permission to use publication in this thesis). The review has been updated for this thesis. All of the literature searches, data extraction and drafting of the manuscript was performed by AJB. Paul Gray also extracted data and all of the authors provided comments and edited the final draft prior to publication.

2.1 Introduction

Although several studies have investigated what happens to cortisol after stroke, (Franceschini et al., 2001, Mitchell, 1997, Olsson, 1999) there are no systematic reviews. It is important to know whether HPA axis activation, or indeed downregulation, is associated with adverse outcomes in patients with stroke because treatments which impact on the HPA axis, and consequently on cortisol, may affect outcomes (such as morbidity (in particular delirium), and mortality).

This systematic review has three main goals. The first is to document comprehensively the evidence on cortisol levels and changes over time following stroke. The second is to determine if there are any associations between cortisol levels and stroke severity. The third is to determine if there are associations between cortisol levels and stroke outcomes- specifically dependency (defined as the degree of functional impairment, measured by, for example, the Modified Rankin Scale (mRS), Barthel Index (BI), Katz Index (KI) or the Glasgow Outcome Scale (GOS)), morbidity and mortality-independent of stroke severity.

2.2 Methods

Searches were conducted, by a single author (AJB), in MEDLINE (from 1966) and EMBASE (from 1980) in April 2013 and PsychINFO in July 2013 and updated in May 2015. Searches used the keywords "stroke" and "cortisol" and their synonyms and were not limited by language (appendix 1). Well-validated search strings, including more than 50 terms, were used to perform the search. Where possible, translations of papers were obtained (possible for one Chinese, one Russian and two Spanish papers, not possible for one Polish, one Bosnian and one Serbian paper). Results were exported to EndNote X4 and duplicates removed. Every title and abstract was read and full texts for all potentially relevant papers were obtained. Inclusion and exclusion criteria were then applied. Reference lists of the included papers and relevant review articles were scrutinized for further references.

2.2.1 Inclusion criteria

1. Full text publication in peer-reviewed journal; 2. Recruited 10 or more participants after stroke (ischaemic, haemorrhagic or subarachnoid hemorrhage (SAH)), older than 18 years; 3. Reported cortisol levels (measured in blood, saliva, cerebrospinal fluid (CSF) or urine) at least once in the first year following stroke.

2.2.2 Exclusion criteria

1. Published only in abstract form; 2. Contained no primary data (for example reviews, editorials); 3. Dissertations or case reports; 4. Did not report data for stroke participants separately from other participants.

2.2.3 Quality Assessment

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was used to assess quality (von Elm et al., 2008). This consists of 22 items, with each item scoring 1 point, thus the maximum score is 22 points. One author (AJB) rated all of the studies (appendix 2).

2.2.4 Data Extraction

Data were extracted using standardized data collection forms. Data concerning study design, participant characteristics and outcome measures was extracted. Two authors (AJB and Paul Gray) independently extracted data for included studies. Any uncertainties were discussed with a third reviewer (Gillian Mead).

2.2.5 Data Synthesis

The data lacked homogeneity, in particular with respect to the timing and method of cortisol measurement, and so were not suitable for meta-analysis. Results were tabulated and summaries of each study were presented (table 2.1).

2.3 Results

11 601 titles and abstracts were scrutinized, and 106 full texts were retrieved (Figure 2.1). Fifty-three studies recruiting 2665 participants (median 36, range 10-281) met the inclusion criteria. The mean participant age range was from 47 years (absolute range 25-69)(Poll et al., 2010) to 80 years (absolute range 75-92)(O'Neill et al., 1991). The proportion of males ranged from 13% (Weant et al., 2008) to 92% (Harney et al., 1993). Twenty-nine studies used a longitudinal methodology and the remaining 24 were cross-sectional studies. Fifty-one of the studies recruited participants from hospital (Szczudlik et al., 2004, Jenkins et al., 1969, Anne et al., 2007, Bendel et al., 2008, Christensen et al., 2004b, Christensen et al., 2004a, Davalos et al., 1996, Espiner et al., 2002, Fassbender et al., 1994, Gustafson Y, 1993, Harney et al., 1993, Johansson et al., 2000, Marklund et al., 2004, Michalaki et al., 2010, Neidert et al., 2011, O'Neill et al., 1991, Poll et al., 2010, Reding et al., 1985, Schwartz et al., 2004, Slowik et al., 2002, Theodoropoulou et al., 2006, Urra et al., 2009, Zierath et al., 2011, Ahmed et al., 2004, Dimopoulou et al., 2005, Dziedzic et al., 2012, Elwan et al., 1990, Harms et al., 2011, Korsic et al., 1990, Lueken et al., 2009, Mangieri et al., 2003, Olsson et al., 1989, Olsson et al., 1992, Parenti et al., 2011, Selakovic et al., 2002, Olsson, 1990, Weant et al., 2008, Murros et al., 1993, Feibel et al., 1977, Zhao et al., 1989, Lanterna, 2013, Back, 2015,

Tu, 2013, Terroni, 2015, Wahab, 2015), and two studies did not specify source of participants (Giordano et al., 2005, Burd and Sergeeva, 1981). Finally, twelve of the studies recruited only first ever stroke (Anne et al., 2007, Bendel et al., 2008, Harney et al., 1993, Murros et al., 1993, Slowik et al., 2002, Theodoropoulou et al., 2006, Ahmed et al., 2004, Szczudlik et al., 2004, Lueken et al., 2009, O'Neill et al., 1991, Dziedzic et al., 2012, Terroni, 2015), eight studies recruited both first ever and recurrent strokes (Christensen et al., 2004a, Christensen et al., 2004b, Davalos et al., 1994, Gustafson Y, 1993, Neidert et al., 2011, Urra et al., 2009, Zierath et al., 2011, Harms et al., 2011) and the remaining twenty-eight studies did not specify this (Olsson, 1990, Dimopoulou et al., 2005, Espiner et al., 2002, Fassbender et al., 1994, Feibel et al., 1977, Giordano et al., 2005, Jenkins et al., 1969, Johansson et al., 2000, Marklund et al., 2004, Michalaki et al., 2010, Poll et al., 2010, Reding et al., 1985, Schwarz et al., 2003, Elwan et al., 1990, Korsic et al., 1990, Mangieri et al., 2003, Olsson et al., 1989, Olsson et al., 1992, Parenti et al., 2011, Selakovic et al., 2002, Weant et al., 2008, Zhao et al., 1989, Burd and Sergeeva, 1981, Lanterna, 2013, Back, 2015, Tu, 2013, Wahab, 2015)

Table 2.1 Characteristics of Studies Included in Systematic Review (listed alphabetically).

Longitudinal Studies:

First Author, Year and Country of Origin	Number of Participants	Mean Age (Y)	Type of Stroke	Cortisol Measurement	Mean Blood Cortisol Level at Baseline, ±SD * (nmol/l)	Association Between Cortisol and Outcome	Correlation Coefficient, Between Cortisol and Outcome (when available)	Study Quality (STROBE 0-22)
Anne 2007 Finland	51	67	Cerebral infarction	Blood	600±200 if dead at 6 months 400±200 if alive at 6 months	Mean cortisol on day 2 and 7 significantly correlated to stroke severity, mRS and mortality	Severity r = 0.44 mRS r = 0.37 Mortality RR= 5.4 am and 5.8 pm	20
Bendel 2008 Finland	30	52	Subarachnoid haemorrhage	Blood (SST)	790±300	Serum cortisol not associated with bleeding severity	NR	20
Christensen 2004 Denmark	172	74	Cerebral infarction (90%) and haemorrhage (10%)	Blood	550	Serum cortisol associated with stroke severity and positive correlation with higher mRS and mortality	Severity r = 0.45 mRS r = 0.18 Mortality OR = 1.9	21
Davalos 1996 Spain	104	66	Cerebral infarction and haemorrhage	Blood and urine	Figures not stated	High free urinary cortisol predicted poor outcome, independent of age, sex and nutritional status at admission	NR	18

Espiner 2002 New Zealand	18	54	Subarachnoid haemorrhage	Blood	520	No data	NR	19
Fassbender 1994 Germany	23	72	Cerebral infarction	Blood	Figures not stated	Cortisol not correlated with stroke severity or with delirium	NR	18
Feibel 1977 USA	65	62	Cerebral infarction, brainstem infarction and subarachnoid haemorrhage	Blood	440 CI 340 BI 717SAH	High cortisol correlated with hypertension and disability	NR	17
Giordano 2005 Italy	32	52	Subarachnoid haemorrhage	Blood	Figures not stated	No data	NR	12
Gustafson 1993 Sweden	83	75	Cerebral infarction	Blood (DST)	450	High cortisol correlated with delirium	NR	21
Harney 1993 USA	12	61	Cerebral Infarction	Blood (DST)	Figures not stated	Abnormal DST associated with depression at 1 week	Depression r = 0.49	17
Huttner 2013 Germany	20	68	Cerebral haemorrhage	Blood	483	No data	NR	19
Jenkins 1969 UK	18	52	Subarachnoid haemorrhage	Blood	535	No data	NR	12
Johansson 2000 Sweden	12	74	Cerebral infarction	Blood	500	Cortisol levels correlated significantly to the severity of paresis	Severity -0.68 to -0.73	18
July 2012 Indonesia	44	52	Subarachnoid haemorrhage	Blood	632 normal ECG 803 abnormal ECG	High morning cortisol levels are associated with ECG abnormalities	ECG abnormality (day 2) OR = 2.56 ECG abnormality (day 4) OR = 1.08	14

Lanterna 2013 Italy	26	54	Subarachnoid haemorrhage	Blood	610	Low cortisol levels associated with poor outcome	NR	17
Laures-Gore 2012 USA	31	57	Cerebral Infarction	Saliva	Not applicable	No relationship between cortisol levels and aphasia severity	Aphasia r = 0.26	17
Marklund 2004 Sweden	88	71	Cerebral infarction	Blood	450	High cortisol levels correlated with severe functional impairment, disorientation and mortality	NR	20
Michalaki 2010 Greece	10	69	Cerebral infarction	Blood (SST)	760	No data	NR	18
Murros 1993 Finland	101	61	Cerebral infarction	Blood	590	Cortisol levels correlated significantly to the severity of paresis (on day 1 and at 3 months) and to the mRS	Severity r = 0.41 (day 1), r = 0.22 (3 months) mRS 0.25	18
Neidert 2011 Switzerland	281	68	Cerebral infarction	Blood	480	Cortisol levels correlated positively with functional outcome and mortality	Functional outcome OR = 1.0 Mortality OR = 1.62	21
O'Neill 1991 UK	23	80		Blood	306 good outcome 752 poor outcome	Cortisol levels independently related to outcome	NR	20

Poll 2010 Germany	22	47	Subarachnoid haemorrhage	Blood	540	Abnormal cortisol (elevated baseline and loss of diurnal rhythm) correlated with lower GCS , longer ICU stay and less favorable outcome	GCS r = -0.56 LOS (ICU) 0.65 Outcome r = -0.67	19
Reding 1985 USA	75	68	Cerebral infarction	Blood (DST)	Figures not stated	Abnormal DST associated with higher depression scores and more severe strokes	NR	16
Schwartz 2003 Germany	22	58	Cerebral infarction	Blood	270	Cortisol not related to outcome	NR	17
Slowik 2002 Poland	70	69	Cerebral infarction	Blood	Figures not stated	Cortisol correlated with stroke severity (-0.42) , and associated with higher mortality rates	Severity -0.42	18
Theodoropoulou 2006 Greece	17	No data	Cerebral infarction	Blood	230	No correlation between cortisol and stroke severity or outcome	NR	15
Urra 2009 Spain	46	74	Cerebral infarction and haemorrhage	Blood	Figures not stated	Cortisol was positively correlated with the NIHSS score (0.31)	NIHSS 0.31	19
Zetterling (Zetterling et al., 2011) 2011 Sweden	55	59	Subarachnoid haemorrhage	Blood	1119 (Median)	Cortisol not related to outcome	NR	18
Zierrath 2011 USA	111	57	Cerebral infarction	Blood	Figures not stated	Cortisol positively correlated with stroke severity (0.72)	Severity 0.72	21

Cross-sectional Cohort Studies (listed alphabetically)

First Author and Year	Number of Participants	Mean Age (y)	Type of Stroke	Cortisol Measurement	Mean Blood Cortisol at Baseline (nmol/l)	Association Between Cortisol and Outcome	Correlation Coefficient, Cortisol and Outcome (when available)	Study Quality (STROBE 0-22)
Ahmed 2004 Sweden	53	66	Cerebral infarction	Saliva	Not applicable	Mean cortisol positively correlated with blood pressure	NR	21
Atanassova(Atanassova et al., 2009) 2009 Bulgaria	33	58	Cerebral infarction	Blood	484	No data	NR	18
Back 2014 Denmark	16	70	Cerebral infarction	Blood	213	No data	NR	16
Burd (Burd and Sergeeva, 1981)1952 Russia	31	NA	Cerebral infarction and haemorrhage	Blood	Not stated	No data	NR	7
Dimopoulou 2005 Greece	33	57	Cerebral infarction and haemorrhage	Blood (SST)	410	No data	NR	17
Dziedzic 2011 Poland	59	58	Cerebral infarction	Blood	590 lower tertile 590 middle tertile 550 upper tertile	High cortisol associated with low serum albumin	NR	15
Elwan 1990 Egypt	51	55	Cerebral infarction	Blood and CSF	12.29 µg%	No data	NR	6

Finklestein 1982 USA	25	72	Cerebral infarction and haemorrhage	Blood (DST)	Not applicable	Abnormal DST associated with depression	NR	14
Harms 2011 Germany	66	72	Cerebral infarction	24 hour urine	Not applicable	Cortisol positively correlated with stroke volume but not severity	Stroke volume 0.32	21
Korsic 1990 Croatia	28	68	Cerebral infarction and haemorrhage	24 hour urine	Not applicable	Cortisol positively associated with mortality	NR	13
Lueken(Lueken et al., 2009) 2009 Germany	32	57	Cerebral infarction	Saliva	Not applicable	No data	NR	20
Mangieri (Mangieri et al., 2003)2003 Brazil	35	52	Subarachnoid haemorrhage	Blood	870	No data	NR	14
Olsson 1989 Sweden	62	75	Cerebral infarction	Blood (DST)	440	High cortisol post DST associated with disorientation but not associated with limb paresis or depression	NR	18
Olsson 1990 Sweden	20	78	Cerebral infarction	Blood (DST) and 24 hour Urine	Figures not stated	Cortisol predicted functional outcome and correlated with presence of limb paresis and disorientation	Paresis 0.59 Disorientation 0.41	19

Olsson 1992 Sweden	16	71	Cerebral infarction	Blood (SST and DST)	390	Abnormal DST correlated with presence of limb paresis and delirium	Paresis 0.62 Delirium 0.66	18
Parenti 2011 Italy	60	60	Subarachnoid haemorrhage	Blood	660	Cortisol positively correlated with Fisher's scale	Severity 0.43	19
Selakovic(Selakovic et al., 2002) 2002 Yugoslavia	53	No data	Cerebral infarction	CSF	Not applicable	No data	NR	16
Shin 2011 Korea	25	56	Subarachnoid haemorrhage	Saliva	Not applicable	Nighttime cortisol negatively correlated with Fisher CT grade	NR	17
Szczudlik 2004 Poland	22	61	Cerebral infarction	Blood	Figures not stated	Cortisol positively correlated with stroke severity	Severity -0.63	16
Terroni 2015 Brazil	36	51	Cerebral infarction	Saliva	0.381	Cortisol positively correlated with anhedonia	NR	16
Tu 2013 China	189	66	Cerebral infarction	Blood	422	Cortisol independent predictor of outcome and mortality	Functional outcome 1.32 Mortality 1.65	21
Wahab 2015 Malaysia	58	67	Cerebral infarction	Blood	428	Cortisol positively correlated with age, but not stroke severity, or mortality	NR	14

Weant 2008 USA	16	58	Subarachnoid haemorrhage	Blood (SST)	620 (Median)	Cortisol positively correlated with length of hospital stay and length of ICU stay	NR	20
Zhao 1989 China	37	66	Cerebral Infarction and Cerebral Haemorrhage	Blood	1120 (Haemorrhagic) 900 (Ischemic)	No data	NR	13

BI, Brainstem Infarction , CI, Cerebral Infarction, DST, Dexamethasone Suppression Test, GCS, Glasgow Coma Scale, ICU, Intensive Care Unit, LOS, Length of Stay, mRS, modified Rankin Scale, NIHSS, National Institutes of Health Stroke Scale, NR, not reported, RR, Risk Ratio, SST, Short Synacthen Test, SD, Standard Deviation, UK, United Kingdom, USA, United States of America, Y, Years.

*Baseline cortisol refers to the first random cortisol sample taken after stroke.

2.3.1 Measurement of cortisol

Of the 53 included studies, 45 (n=2430) measured cortisol in blood (table 1). Thirteen studies (n=512) used either the dexamethasone suppression test (DST) or short synacthen test (SST) (which included a random, pre-test, measure of blood cortisol), 18 (n=1419) measured cortisol in blood in the early morning and 13 (n=532) measured diurnal cortisol levels. The remaining eight measured salivary (n=182), urinary (=94) and/or cerebrospinal fluid (CSF) (n=53) cortisol. There was variability in time from stroke to first sample being taken (median 1 day, interquartile range (IQR) 1-3 days, range 0-111 days).

2.3.2 Cortisol levels and changes over time following stroke

Cortisol after stroke

Cortisol levels in blood (n=2242) at baseline (i.e. at the time of recruitment) following admission to hospital ranged from 200 nmol/L (Anne, Juha et al. 2007) to 1120 nmol/L (Zhao, Liu et al. 1989). The majority of studies (28 studies, n =1562) found that cortisol levels were high (outwith the reference range) within the first week after stroke onset. Eight studies compared baseline cortisol levels to those in controls. The majority of controls were healthy age-matched individuals; one study included both healthy controls and controls admitted with non-stroke acute medical conditions (Olsson, Astrom et al. 1989). Three of these studies found no significant difference (n=187) (Olsson, Marklund et al. 1992, Gustafson Y 1993, Marklund, Peltonen et al. 2004), four (n=163) (Zhao, Liu et al. 1989),(Burd and Sergeeva 1981, Olsson, Astrom et al. 1989, Atanassova, Terzieva et al. 2009) found significantly higher cortisol in stroke patients and one study (n=25) found that male, but not female, stroke patients had significantly higher cortisol compared to controls (Elwan, Abdallah et al. 1990).

Changes in cortisol over time

Thirteen studies (n=567) measured cortisol at two or more time points on different (non-consecutive) days and reported changes over time. Five studies (n= 264) reported that cortisol fell (Davalos, Ricart et al. 1996, Espiner, Leikis et al. 2002, Marklund, Peltonen et al. 2004, Giordano, Aimaretti et al. 2005, Poll, Bostrom et al. 2010), with two of these (n=106) (Espiner, Leikis et al. 2002, Marklund, Peltonen et

al. 2004) reporting cortisol levels within the reference range at follow up (4 days and 2 weeks respectively) in all participants. One study (n=22) reported low (below the reference range) serum cortisol in all subjects after stroke for the duration of the study period (nine days on average)(Schwartz, Carlucci et al. 2004). Two studies found persistent elevation of cortisol over the duration of their study period, one of which studied participants up to day five after stroke (n= 23) and one up to one month after stroke (n= 111) (Fassbender, Schmidt et al. 1994, Zierath, Tanzi et al. 2011). One study reported a peak of cortisol at day 5, with troughs at day 1-2 and day 8 (n=26)(Lanterna 2013). Four studies (n=121) reported that cortisol was within the normal reference range over the entire study period (ranging from seven days to three months).

Dexamethasone Suppression Test (DST) after stroke

Nine studies (n=347) used the DST (median day of first test; day 5 post stroke, IQR 2.8-5.8 days); all reported non-suppression of cortisol in stroke participants (Jenkins, Buckell et al. 1969, Finklestein, Benowitz et al. 1982, Reding, Orto et al. 1985, Olsson, Astrom et al. 1989, Olsson 1990, Olsson, Marklund et al. 1992, Gustafson Y 1993, Harney, Fulton et al. 1993, Terroni 2015). This persisted over time in the three studies that repeated the test (median day of second test day 17.5 post stroke, IQR 3.5-28 days).

Diurnal variation in cortisol after stroke

Eleven studies (nine using blood and two using saliva) analyzed diurnal variation in cortisol. Six studies measured cortisol twice during a 24 hour period (morning and evening) and the remainder took measurements four times during a 24 hour period (generally early morning, mid-morning, early evening and just before bed). Five (n=196) found that diurnal variation was lost in those with more severe strokes (as determined by a validated scoring scale, for example the Scandinavian Stroke Scale) but preserved in those with more minor strokes (Murros, Fogelholm et al. 1993, Szczudlik, Dziedzic et al. 2004, Anne, Juha et al. 2007, Atanassova, Terzieva et al. 2009, Poll, Bostrom et al. 2010). Two studies (n=82) (Johansson, Ahren et al. 2000, Slowik, Turaj et al. 2002) found that diurnal variation was lost in those with high baseline cortisol, but did not assess whether this was associated with stroke severity. One study found enhanced diurnal variation in those diagnosed with anhedonia

(n=36) (Terroni 2015). One study (n=22) (Schwarz, Schwab et al. 2003) found that diurnal variation was lost between days one and seven after stroke, and one study found that over half (17/22) of their participants had an abnormal diurnal variation in the first week after stroke, but at one month this change persisted in only two participants(Jenkins, Buckell et al. 1969). Finally, one study which recruited only those with mild stroke (n=17) (Theodoropoulou, Metallinos et al. 2006) found that diurnal variation was preserved in all participants.

2.3.3 Associations between cortisol and stroke severity

Eighteen studies investigated the associations between cortisol and stroke severity (of these 18, four included only those with a SAH).

Ischaemic or haemorrhagic stroke

Stroke severity was measured using a variety of rating scales, however the most frequently used scales were the National Institutes of Health Stroke Scale (NIHSS) and the Scandinavian Stroke Scale (SSS). Twelve studies (n=1155) found a statistically significant correlation between elevated cortisol levels and more severe strokes(Reding, Orto et al. 1985, Olsson 1990, Olsson, Marklund et al. 1992, Murros, Fogelholm et al. 1993, Fassbender, Schmidt et al. 1994, Slowik, Turaj et al. 2002, Christensen, Boysen et al. 2004, Anne, Juha et al. 2007, Urra, Cervera et al. 2009, Neidert, Katan et al. 2011, Zierath, Tanzi et al. 2011, Tu 2013) and one (n=25) found an association (not statistically significant)(Finklestein, Benowitz et al. 1982) . The remaining five (n=262) found no association (Olsson, Astrom et al. 1989, Theodoropoulou, Metallinos et al. 2006, Harms, Reimnitz et al. 2011, Dziedzic, Pera et al. 2012, Wahab 2015).

Subarachnoid haemorrhage

Subarachnoid haemorrhage severity was measured using the Hunt-Hess score, the Fisher score or the Glasgow Coma Scale score. Of the four studies involving only participants with a SAH, one (n=51) found a small correlation between higher morning cortisol levels and Glasgow Coma Scale [22,] one (n=60) found a correlation between higher cortisol and the Fisher scale(Parenti, Cecchi et al. 2011), one (n= 25) found a negative correlation between cortisol concentrations and the Fisher CT

grade(Shin, Joo et al. 2011) and one (n=30) found no correlation between urinary cortisol and the Hunt-Hess score(Bendel, Koivisto et al. 2008).

2.3.4 Associations between cortisol and stroke outcome (including dependency, morbidity and mortality)

Cortisol, dependency and length of stay

Twelve studies (n=1131) reported the association between cortisol and dependency, of which nine (n=1011) found that higher cortisol was associated with more dependency(Feibel, Hardy et al. 1977, Murros, Fogelholm et al. 1993, Johansson, Ahren et al. 2000, Slowik, Turaj et al. 2002, Christensen, Boysen et al. 2004, Marklund, Peltonen et al. 2004, Dimopoulou, Kouyialis et al. 2005, Neidert, Katan et al. 2011, Tu 2013), and three studies (n=120) did not(Reding, Orto et al. 1985, Fassbender, Schmidt et al. 1994, Schwarz, Schwab et al. 2003) (table 1). Of the nine studies which found an association, eight measured cortisol within 24 hours of stroke onset, as did two out of the three studies which found no association. Three studies reported the relationship between cortisol and length of stay; two (n=38) reported that higher cortisol was associated with a longer length of intensive care unit stay(Poll, Bostrom et al. 2010) (Weant, Sasaki-Adams et al. 2008), and one of these also found an association between higher cortisol and overall length of stay(Weant, Sasaki-Adams et al. 2008). The third study (n=25) found no relationship between cortisol and length of hospital stay, however this study was in a rehabilitation hospital, and so cortisol was measured relatively late (mean of 37 days) after stroke(Finklestein, Benowitz et al. 1982).

Cortisol and morbidity

Five studies examined the relationship between cortisol and delirium. Three (n=187) found a correlation between elevated cortisol and delirium(Olsson, Marklund et al. 1992, Gustafson Y 1993, Marklund, Peltonen et al. 2004), one (n=20) found a non-significant trend towards this association(Olsson 1990) and one (n=23) found that a high adrenocorticotrophic hormone level (ACTH) was associated with delirium(Fassbender, Schmidt et al. 1994) (see table 1 for summary of effect sizes). Five studies examined the relationship between cortisol and depression. Three studies (n=117) found a correlation between an abnormal DST and depression(Finklestein,

Benowitz et al. 1982, Reding, Orto et al. 1985, Theodoropoulou, Metallinos et al. 2006) one (n=12) found a non-significant association between higher cortisol and depression(Harney, Fulton et al. 1993) and one (n=62) found no relationship(Olsson, Astrom et al. 1989). Finally, one (n=66) study investigated the relationship between cortisol and infection and found a positive correlation(Harms, Reimnitz et al. 2011), two studies (n=131) investigated the relationship between cortisol and blood pressure and also found a positive correlation(Feibel, Hardy et al. 1977, Ahmed, De La Torre et al. 2004) and one study(n=44) found an association between electrocardiographic abnormalities after SAH and elevated morning cortisol levels(Djurdjevic, Jovanovic et al. 2010).

Cortisol and mortality

Of the eleven studies (n=1084) (Feibel, Hardy et al. 1977, Korsic, Brinar et al. 1990, O'Neill, Davies et al. 1991, Murros, Fogelholm et al. 1993, Slowik, Turaj et al. 2002, Christensen, Boysen et al. 2004, Marklund, Peltonen et al. 2004, Anne, Juha et al. 2007, Weant, Sasaki-Adams et al. 2008, Neidert, Katan et al. 2011, Tu 2013) which examined the relationship between cortisol and mortality, all found that elevated cortisol was associated with increased mortality (see table 1 for summary of effect sizes); this was not statistically significant in two of the studies (n=39)(O'Neill, Davies et al. 1991, Weant, Sasaki-Adams et al. 2008).

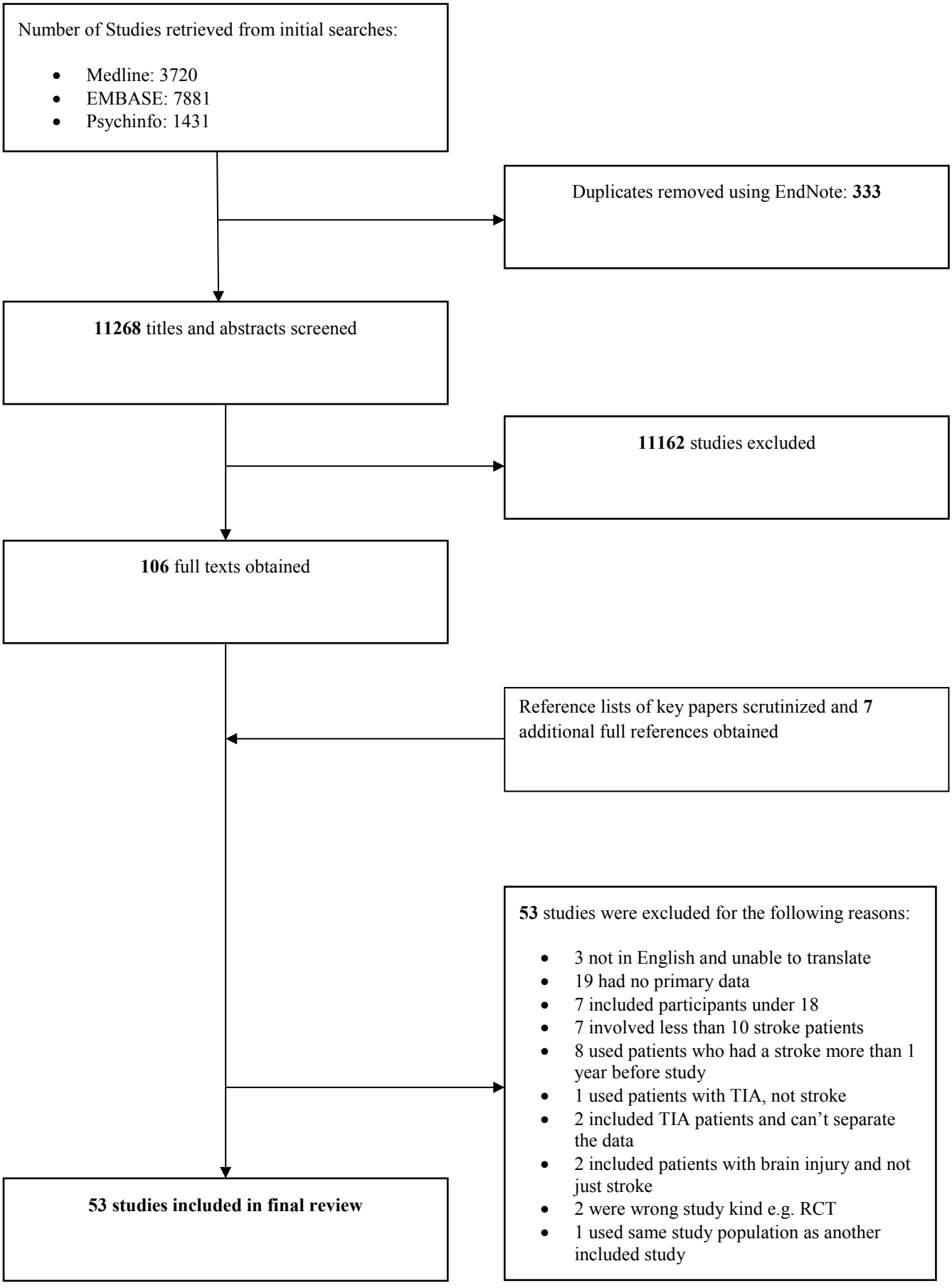
Cortisol and outcome, independent of stroke severity

Only six studies adjusted for stroke severity when examining the relationship between cortisol and outcome. Four studies (n=693) (Christensen, Boysen et al. 2004, Anne, Juha et al. 2007, Neidert, Katan et al. 2011, Tu 2013) found that cortisol was independently associated with death after stroke and three out of four (n=642) also found higher cortisol to be independently associated with poorer functional outcome (Christensen, Boysen et al. 2004, Neidert, Katan et al. 2011, Tu 2013). The remaining two studies (n=215) found that cortisol was not an independent predictor of outcome after adjusting for stroke severity(Davalos, Ricart et al. 1996, Zierath, Tanzi et al. 2011).

2.3.5 Methodological Quality

The STROBE score ranged from 6-21, with a median score of 18 (appendix 2). The lower quality studies (particularly those with a STROBE score of less than 10) did not report the relationship between cortisol and stroke outcome, and so these papers will have had little influence on the main conclusions of this review

Figure 2.1 Schema of systematic review



2.4 Discussion

This is the first systematic review of studies of cortisol levels in relation to stroke severity and outcomes. Fifty-three studies met our inclusion criteria. The methodological quality of these studies was generally high.

Summary of main findings

Cortisol levels were high (meaning above the reference range (absolute range 200 nmol/L(Anne, Juha et al. 2007) to 1120 nmol/L(Zhao, Liu et al. 1989))) in the first seven days after stroke onset. Those studies including participants with less severe strokes (not requiring critical care) found a decrease in cortisol over the first week following stroke. By three months cortisol levels were generally in the normal reference range, for all strokes regardless of severity. We are not able to conclude what the trajectory of cortisol is between these two time points (seven days and three months) as few studies investigated this. Diurnal variation in cortisol appears to be lost in those with more severe strokes, but is preserved in those with more minor strokes.

Elevated cortisol levels were correlated with increased stroke severity in the majority of studies that explored this association. Studies tended to find that elevated cortisol levels were associated with higher dependency, length of hospital stay, depression, delirium and mortality. The association between cortisol levels and delirium was examined in five of the included studies. Overall the majority (three out of five studies, n=117) found an association between higher cortisol levels and development of delirium, although one study found no association (n=62) (Fassbender, Schmidt et al. 1994) however the major limitation to all of these five studies is the small sample size (n ranged from 12-88). None of these five studies reported a power calculation, and it is unlikely that any of them were adequately powered (individually at least) to draw firm conclusions about associations between stroke and delirium. However, they do provide a signal that there might be an association, which requires further exploration. It is important to note that even if an association were shown in larger studies, we do not know the direction of causality- whether stroke precipitates high cortisol levels which causes delirium, or whether pre-existing high cortisol precipitates stroke which then causes delirium, for example.

Discussion about the included studies

There are some limitations in the included studies: Only one of the included studies reported sample size calculations and several studies only measured cortisol on one occasion rather than exploring changes over time, meaning that short-term physiological stressors such as acute illness may have contributed significantly to the cortisol levels reported. Furthermore, all the studies recruited participants from a hospital setting, meaning that results may not be applicable to those with minor strokes. Thirteen studies (Jenkins, Buckell et al. 1969, Finklestein, Benowitz et al. 1982, O'Neill, Davies et al. 1991, Davalos, Ricart et al. 1996, Johansson, Ahren et al. 2000, Christensen, Boysen et al. 2004, Christensen, Johannesen et al. 2004, Szczudlik, Dziedzic et al. 2004, Theodoropoulou, Metallinos et al. 2006, Lueken, Leisse et al. 2009, Michalaki, Margeli et al. 2010, Wahab 2015) included only those able to provide informed consent, meaning that those with aphasia or delirium would have been excluded. This could have reduced the generalizability of the findings particularly as delirium has been found to be associated with higher cortisol (Gustafson Y 1993). It is also possible that the results presented are confounded by unmeasured variables, for example an association between cortisol and stroke may reflect a causal relationship with a hormone, neurotransmitter or other physiological parameter which was not measured in any of the included studies. Finally, it must be acknowledged that the STROBE statement, used to assess quality of the included studies, gives equal weight to all aspects of quality, such that providing information about the source of funding for the work is given the same marks as providing appropriate statistical analysis, when although both of these points are important, the analysis is (arguably) more crucial to the overall quality of the paper.

Strengths of the review

This systematic review has several strengths. The protocol had pre-defined inclusion and exclusion criteria. Screening and data extraction was performed independently by two authors, reducing the risk of transcription and data extraction error or omission. Systematic search strategies were used, and so it is unlikely that relevant articles were missed.

Limitations of the review

Some limitations of this review should be acknowledged. I did not include abstracts from conference proceedings, however this was deliberate crucial details are often missing from these publications. Only six abstracts of conference proceedings would have met the inclusion criteria. From the limited information available in these proceedings, interestingly, it would appear than none of them reported negative findings, with four out of six reporting abnormalities in cortisol after SAH(Djurdjevic, Jovanovic et al. 2010), (Takala, Laukka et al. 2013), (Hannonl, Behan et al. 2012), (Tolli, Bellander et al. 2010) one reporting evening cortisol levels above the reference range after ischaemic stroke(Lee, Kim et al. 2011) and one reporting an association between high cortisol and stroke severity(Hall, Malik et al. 2010). Ischaemic and haemorrhagic strokes, including SAH, were included in the review. It could be argued that as SAH has a different aetiology and risk factor profile to ischaemic and haemorrhagic stroke, it should have been excluded; however I have taken care to report the findings from those studies including SAH separately. Furthermore, SAH does have several factors in common with other stroke types, for example sudden onset of disease and long-term neurological sequelae. I was not able to perform a meta-analysis because the studies were too heterogeneous, particularly with respect to the timing and method of cortisol sampling. Finally, publication bias may have favored publication of those papers showing a positive association between cortisol and stroke, leading us to overestimate the strength of the association.

Previous non-systematic narrative reviews have found a correlation between cortisol and functional impairment and mortality after stroke (Mitchell 1997, Olsson 1999, Franceschini, Tenconi et al. 2001), however two of these previous papers included discussion about cortisol after stroke only as part of a broader review of endocrine or of cognitive changes(Olsson 1999, Franceschini, Tenconi et al. 2001) and the third, whilst providing a more extensive overview, was published in 1997, and consequently includes only 17 studies(Mitchell 1997). This systematic review provides a more comprehensive overview of all studies to date and synthesizes the evidence.

Overall, if cortisol dysregulation was shown to be an independent predictor of poor outcome after stroke, even after correcting for stroke severity, this would provide

justification for further investigating the mechanism of this. Whilst I have found some evidence of an independent association between cortisol and functional outcome, and between cortisol and mortality after stroke, we do not know what the direction of causality is (no studies were able to measure cortisol pre-stroke and so it is possible that those with poorer outcomes may have had higher cortisol levels before stroke onset, for example). Further larger studies designed to unpick the complex relationship between the HPA axis and stroke are required before, for example, trials to target cortisol dysregulation after stroke could be justified.

2.5 Conclusions

Cortisol levels are high for at least seven days after stroke and are within the normal range in the majority of people by three months. Elevated cortisol after stroke is associated with greater dependency, morbidity and mortality. However, there is currently insufficient evidence to conclude that these relationships are independent of stroke severity. Understanding the mechanism underlying these relationships may allow development of therapeutic interventions to improve outcomes after stroke and merits further investigation.

Chapter 3: Service User Group

3.1 Introduction

Consulting service users about proposed research projects is a way of ensuring that the study is not only acceptable to participants, but also of ensuring that the research questions are truly relevant to them. Furthermore, service users have been found to be a good way of disseminating research results into a public domain, and can offer valuable advice on how to present these results in a way that is accessible to the lay public (Hanley 2004). However, user groups have attracted some criticism and scepticism from the scientific community. For example, one major criticism levelled at user groups made up of volunteers from a large group of stroke survivors, is that they tend to be those who have had milder strokes and are from higher socio-economic groups, and therefore not truly representative of the stroke survivor population as a whole (Hanley 2004). However, it must be acknowledged that it is not reasonable to expect a small number of people to be truly representative of the target population (in this instance, stroke survivors), and indeed in many instances it is not necessary for them to be so. Rather it is useful to see other perspectives despite the fact that they may not be representative of the whole study population (Hanley 2004).

3.2 Methodology

A user group was convened prior to the study commencing. The group comprised of six service users who had recently had a stroke (within the last 12 months) and who were attending a community rehabilitation centre on a weekly basis. The service users were all residents of Edinburgh, but hailed from a variety of areas of the city. The median age of the service users was 64 (range 48-67). The service users were asked if they were happy to participate in the group, prior to my attendance, but were not volunteers taken from a larger pool of service users. The service users were given a verbal briefing about their role as a user group member and the aims of the meetings and were also provided with some written briefing information on the role of a user group and about the study (appendix 3). Two meetings were scheduled in total, one prior to the study commencing and one when the study had been recruiting for one year. Group members were interviewed together, before being given the opportunity

to speak to me individually about the study. The minutes of the meetings were collated and circulated to group members for agreement and action points based on the discussions were then formulated.

3.2.1 The main aims of the first user group meeting were:

1. To find out how the service users view the study information sheets. The questions posed were:
    a. Are the information sheets clear? Do you understand the study and what is being asked of participants
    b. Do you think the study is important?
    c. What aspects of the study do you think participants might find daunting or difficult and how can I best minimise these?
    d. Any other suggestions to improve the acceptability of the study to participants?

2. To find out how the users view recruitment:
    a. When do they think the best time to approach a potential participant is (time of day and time from stroke)
    b. What are the potential barriers to patients agreeing to take part?
    c. How best can researchers overcome these barriers?

3. To determine how users feel about the number and intensity of assessments and follow-up visits
    a. Will the proposed number of assessments be acceptable to stroke participants?
    b. Will the number of follow-up visits required seem onerous, or is the number and time scale about right?

3.3 Results:

3.3.1 Meeting One

Information Sheets:

The group felt that the information sheets were informative and gave a good overview of the study. All felt that they understood the study after reading the sheet. Some commented that the sheet was quite long, and so they would require to be given sufficient time to read this and digest it. They didn't have any specific suggestions with respect to changes to the sheets.

Recruitment:

Service users understood the need to recruit participants in the first few days after stroke. Some of the group felt that being approached about research in the first few days after stroke may be too much to cope with, as they found the experience of having a stroke very overwhelming and difficult to come to terms with. However some of the group members felt that they would have been interested in research even in the early days following stroke, as it would have provided a "welcome diversion" and it "would have been nice to talk to someone" who wasn't discussing direct aspects of clinical care. The group agreed that following a stroke, lots of adjustments had to be made in terms of how they felt about themselves and in particular in terms of confidence. They felt that feeling "useful" as part of a study may help some people. They also were, however, keen to point out that fatigue was a significant feature early after stroke and felt that a researcher should bear this in mind. All agreed that research into memory and thinking after stroke would be a good thing.

Number of assessments/follow-up:

Some of the group felt that the number of follow-up visits in hospital might be too much for some people, depending on how they are feeling. The remainder of the group felt that as each follow-up visit will be quite short, that this would be acceptable.

All agreed that they would be happy to be seen in their own home at 4 months and 1 year. One of the group was keen to point out that planning and organisation is now key to his life as day to day tasks take significantly longer to perform than they used

to. To this end, both a letter (one week prior to the visit) and then a telephone call (one day prior to the visit) to remind participants of the visits were recommended.

General Comments:

The group wondered if feedback will be given to individuals about their performance in memory tests. They felt that if participants performed badly in the testing, this may be another source of worry. They did however agree that if a participant wanted to know the results, they should be given them, but with an explanation that this will be monitored over time and that any on-going problems will be investigated. All agreed that it would be desirable for participants to have access to the study results overall when they are available. A newsletter was felt to be a good way of letting participants knows the results.

Conclusions and action points:

Following the user group meeting, the study protocol was finalised, incorporating suggestions, such as the reminders about follow-up visits, and a plan to provide a newsletter for participants.

3.3.2 Meeting Two

A second user group meeting was convened one year into the study, and four of the original six users were able to participate in this second meeting. The aims of the second meeting were:

1. To discuss how to maximise participant retention in the study
2. To discuss any other suggestions about how to improve the participant experience in the study

Participant retention:

All felt that the topic of the study is of interest and that stroke survivors are definitely concerned about cognition. They all felt that the number of follow-up visits would have an impact on retention, however they agreed that the study seems to have the number about right. Personal feedback about any changes in cognition and appropriate referral if a problem was found were thought to be the key issues which would ensure participants are retained. It was felt that although a newsletter would be interesting to read, it would be more general and so it was felt that this would have less impact on retaining participants. No other specific suggestions were raised in terms of improving retention.

General comments:

The user group did not have any further suggestions about improvements to the study protocol.

Conclusions and action points:

Specific feedback to participants will be give when requested, and the participant's general practitioner will be informed of any concerns regarding cognition, with the participant's permission.

3.4 Discussion:

The user group provided useful insights into how information is presented to participants, both verbally and in written form and also provided some helpful insights into the first days and weeks after stroke and how best to recruit and retain participants at this vulnerable time in their lives. The group provided insights about how to give feedback to participants and the format this might take, and particularly emphasised the value of individual feedback and discussion with the researcher about any ongoing problems with thinking and memory, and how this is a significant attribute of the study.

There are several benefits to involving service users in the planning and implementation of research studies. Service users are able to offer different perspectives to health care professionals, and can help to ensure that issues important to them are prioritised. Service users may also help to ensure that money and resources are not wasted on research which has no relevance and doesn't just measure outcomes that are important to the researchers, but not to patients. Finally, service users can help to disseminate research results by assisting with production of accessible information about the results and also through contacts with other patient groups (Hanley 2004).

Whilst the benefits of including a user group in the design and implementation of studies are acknowledged, it is also important to note that there may be some disadvantages to user groups. For example, it is imperative that although the suggestions of the stroke survivor be incorporated where possible, the scientific rigour of the study is not compromised in doing so. Suggestions of the user group can also lead to ethical and scientific conflicts in protocol design. For example, a study designed to investigate the role of routine oxygen supplementation after stroke found that their user group would prefer to have a waiver of consent for those without capacity rather than proxy consent, as they felt that the doctor looking after them would be better placed to make the decision about study entry, than a family member, who may be more "emotionally involved" with the patient (Ali 2005). This also illustrates the importance of education and guidance for the user group, in particular about the role and remit of the group, before the main meetings are arranged. It is

more costly and time consuming to involve service users in research. Generally, whilst the service users are not paid for their involvement, expenses are met by the research fund. Finally, it is important that consideration is given to selection of the user group as previously discussed.

Chapter 4: General Methods

4.1 Study Design

The study comprised of a cohort of participants admitted to the acute stroke unit at the Royal Infirmary of Edinburgh between October 2012 and March 2014

4.2 Recruitment

Ninety-five participants were recruited from the acute stroke unit at the Royal Infirmary of Edinburgh. The recruitment flow chart is shown in figure 4.1.

Inclusion criteria were:

1. Clinically confirmed stroke (by a stroke physician)
2. Age ≥ 60 years (because patients over the age of 60 have higher rates of delirium)

Exclusion criteria were:

1. Patients with a diagnosis of transient ischaemic attack
2. Subarachnoid haemorrhage (as these patients are generally admitted under the care of the neurosurgeons, not the stroke physicians, and because the underlying aetiology is fundamentally different, i.e. usually an intracranial aneurysm)
3. Other stoke mimic found on brain imaging, such as a tumour
4. Current or recent use (within 6 months) of oral or inhaled corticosteroids
5. Active alcohol withdrawal
6. Inability to speak English prior to stroke

Participants were recruited within 120 hours of stroke onset; for those participants who had their stroke during the night, the time of stroke onset was taken to be the time when they were last seen well. Potential participants (those who met the inclusion criteria) were approached, in alphabetical order, by one of the clinical team and asked if they would like to discuss the study with the research team. I (AJB) then approached those patients who agreed to meet me, and discussed the study and

provided the written study information sheet (appendix 4). Potential participants were then allowed up to 24 hours to read the information and consider whether to take part. I then revisited, having provided sufficient time for them to consider the study, discussed the study, answered any questions and assessed their capacity to provide consent. For those deemed to have the capacity to provide informed consent, a consent form was completed by the participant (appendix 4) For those deemed to lack capacity, the study was also discussed with their next of kin (who were also provided with written information (appendix 4) , and subsequently asked to complete a consent form (appendix 4)). Proxy consent was then obtained for those who provided assent to participate, but who lacked the capacity to provide informed consent themselves (e.g. because of aphasia, delirium or dementia). Participants who lacked capacity at recruitment, and for whom proxy consent was obtained, but who regained capacity during the course of the study (for example because of resolution of delirium or improvement in aphasia) were retained in the study, providing they were agreeable to continue and to sign a consent form.

Participants with aphasia were provided with accessible information sheets (see appendix 5) to aid their ability to understand the study, however the Research Ethics Committee required that if a participant had severe enough aphasia to require the aphasia friendly information, proxy consent must be obtained. If participants were unable to follow basic commands due to a dense receptive aphasia, they were excluded from the study and if any participants remained unable to complete cognitive assessment after 2 weeks due to severe aphasia, they were then excluded from the study.

Participants (and proxy's where relevant) were provided with a copy of their signed consent form, and a copy was also placed in the participants medical notes. A letter was sent out to each participant's General Practitioner (GP) informing them about the study and providing contact information, should any subsequent queries arise.

A summary diagram of the study protocol is shown in figure 4.2.

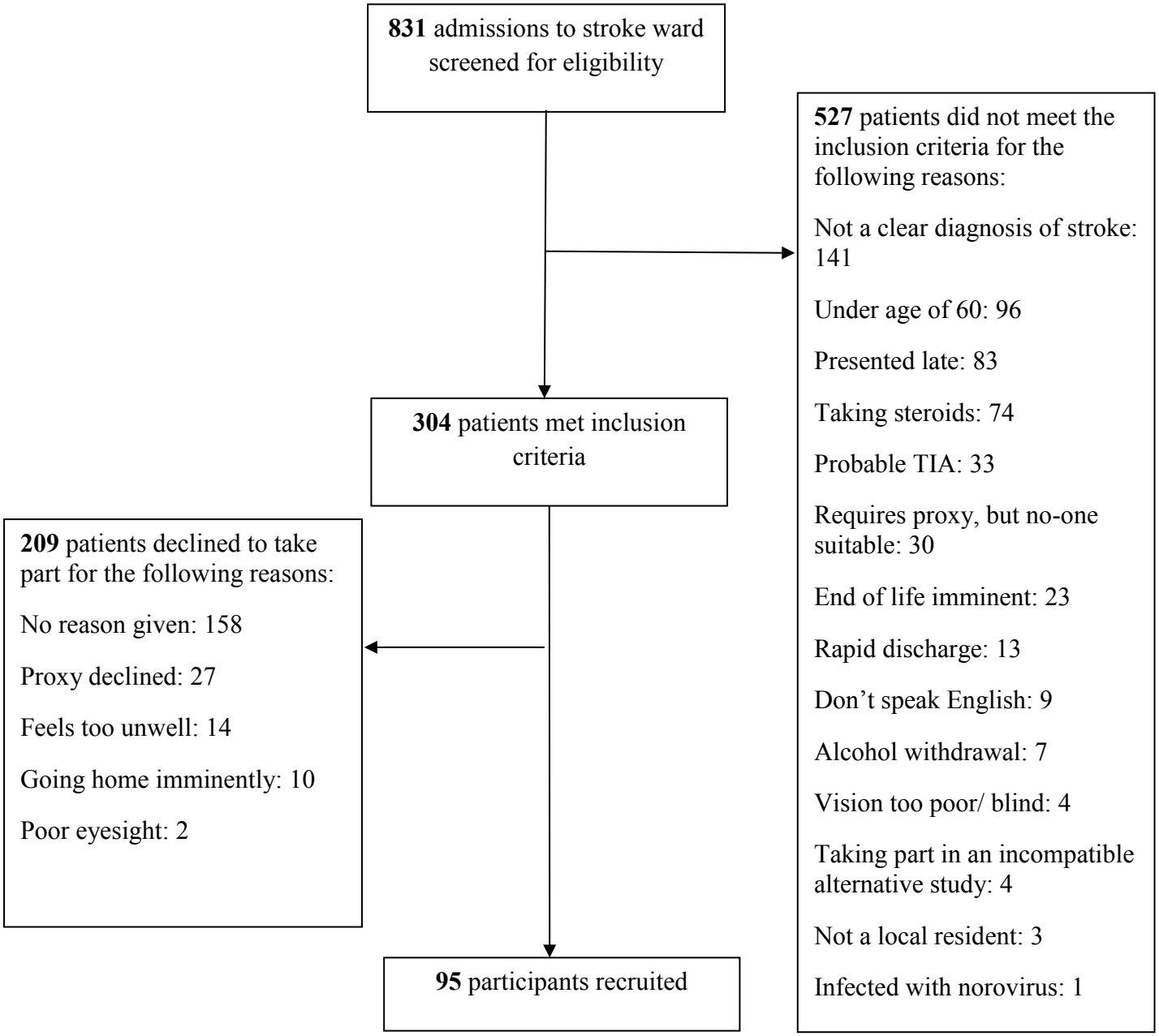


Figure 4.1 Recruitment flow chart

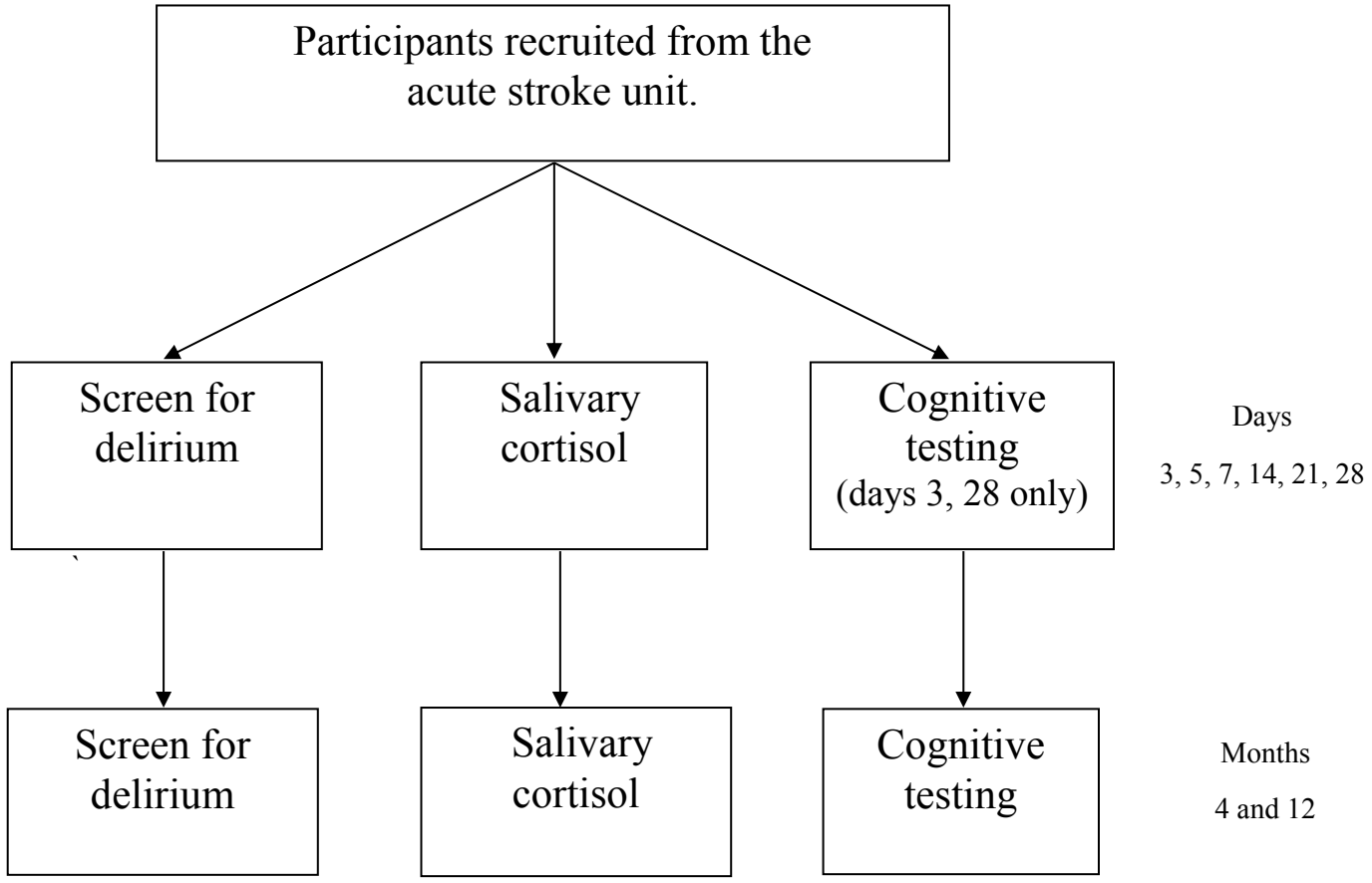


Figure 4.2 Summary of the study protocol

4.3 Baseline data collection

Stroke: The pathological stroke type (ischaemic or haemorrhagic), Oxfordshire Community Stroke Project classification (from clinical assessment performed at time of admission: by convention we used the maximum deficit to rate this) and National Institute of Health Stroke Scale score (for stroke severity, rated by me (AJB), based on the maximum deficit) were documented at the time of recruitment.

Clinical condition: Baseline clinical observations (heart rate, blood pressure, oxygen saturations and temperature) were recorded at the time of recruitment, along with medications and admission blood results. The APACHE II (The Acute Physiology and Chronic Health Evaluation System II (Chisakuta 1990)) score was used as a measure of current illness burden. Past medical history, and current medications (along with any changes made on admission) were extracted from case notes.

4.4 Delirium assessment

Participants were assessed for the presence of delirium after the onset of stroke at days 1, 3, 5, 7, 14, 21 and 28, and also at 4 months and 12 months. Those recruited after day 1 were seen from day 3 or day 5 onwards. Those participants who were discharged from hospital after a short admission were seen as per protocol whilst an in-patient and were then seen at home on day 28 and at 4 months and 12 months. I (AJB) was trained in the diagnosis of delirium, and in the use of the screening tests employed, by experienced raters (AMJM and Dr Roanna Hall). Training involved reading of the manual and guidelines for each of the tests (where available) and then several sessions when I observed experienced raters applying the tests, and finally sessions where I applied the tests under observation by the experienced raters.

The presence and severity of delirium was screened for using the CAM-ICU (Ely 2001), which includes the Richmond Agitation and Sedation Scale (RASS) (Sessler CN 2002), and the Delirium Rating Scale-Revised-98 (Trzepacz PT 2001). Attention was assessed by digit span (forwards and backwards) and a computerised testing

instrument validated for the detection of attentional deficits in delirium (The DEL-box mark 2 (Brown LJE 2012)). A diagnosis of delirium was made based on the DSM-IV criteria (section 1.2.5) using the results of the screening tests to help to inform the diagnosis, and to assess specific features of delirium (such as sleep-wake cycle, psychosis and so on). A diagram to show the delirium assessment procedure is shown in figure 4.3

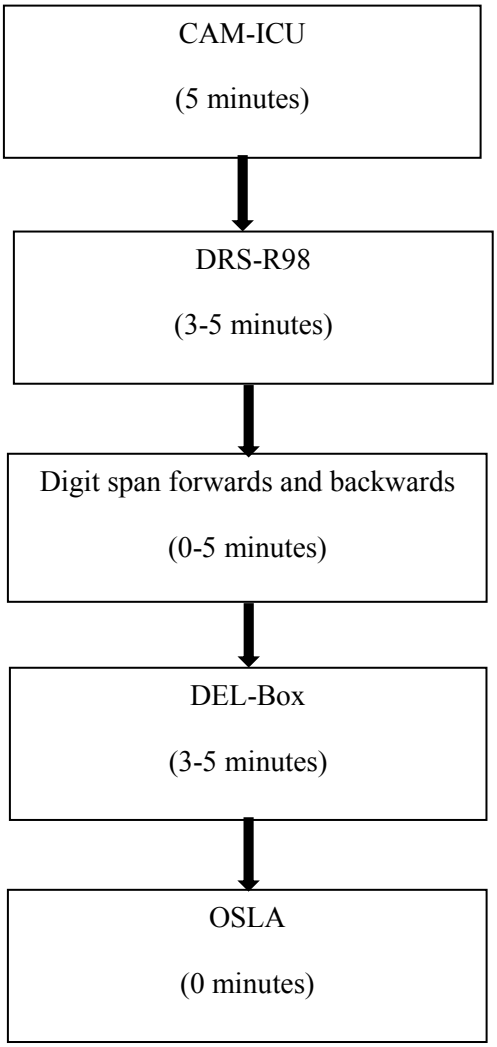


Figure 4.3 Flow chart to show the delirium assessment procedure undertaken at each assessment. The time required per test from the patients perspective is shown in brackets.

4.4.1 The Confusion Assessment Method for the Intensive Care Unit (CAM-ICU)

The CAM-ICU was developed from the original Confusion Assessment Method (Inouye, van Dyck et al. 1990) for use by non-psychiatrists to screen mechanically ventilated patients in the ICU for delirium. These patients are unable to produce speech (sometimes termed 'non-verbal') and thus the tool does not require the ability to verbalise. This tool was therefore selected for use in the acute stroke unit, as it is possible to screen those with aphasia, and it has successfully been used in previous studies of delirium after stroke (McManus 2009). The CAM-ICU is a diagnostic algorithm consisting of four features, which are based on the DSM diagnostic criteria:

1. Acute onset or fluctuating course
2. Inattention
3. Disorganized thinking
4. Altered level of consciousness (assessed using the Richmond Agitation Sedation Scale, RASS, see below)

It is possible to test all of the features in those who are non-verbal, for example participants are requested to squeeze the tester's hand in response to the attention screening examination and are asked to follow a series of physical commands as part of the assessment of disorganised thinking (for example, they are required to hold up 2 fingers on one hand and then to add a third finger to those being held up). Those who have a dense aphasia and are not therefore able to follow these instructions, were excluded from this study, as per the prespecified protocol, although in the participants recruited it was not necessary to exclude anyone for this reason. It is also possible to test the features in those who have a hemiparesis following stroke, as all of the commands can be completed using just one arm. The CAM-ICU has been shown to have a sensitivity of 93-100% and specificity of 98-100%, and a high inter-rater reliability ($\kappa = 0.96$) in the detection of delirium (Ely 2001), although this was in the general ICU population, not specifically after stroke (the gold standard against which the CAM-ICU was rated was a psychiatrist assessing the patient and diagnosing delirium using DSM-IV criteria). Indeed, in non-critically ill hospitalised patients the CAM-ICU has been shown to have poor sensitivity (18%), although very high specificity (>98%) for diagnosis of delirium (Neufeld, Hayat et al. 2011), and so the

CAM-ICU algorithm was not the cornerstone of making a diagnosis of delirium, rather it was used to help inform the diagnosis, based on DSM-IV criteria.

4.4.2 The Richmond Agitation and Sedation Scale (RASS)

The Richmond Agitation and Sedation scale (Sessler CN 2002) is an integral part of the CAM-ICU and is a measure of alertness. It is an observational scale, with a score of 0 equating to the participant being 'alert and keenly responsive'. The scale ranges from -5 (unrousable) to +4 (combative). The RASS has been shown to have good sensitivity (82%) and specificity (85%) for diagnosis of delirium (compared with the gold standard of a psychiatrist making a diagnosis based on DSM-IV criteria) in the general elderly (aged >65) Emergency Department population (Han, Vasilevskis et al. 2015).

4.4.3 The Delirium Rating Scale-Revised-98 (DRS-R-98)

The DRS-R-98 is a 16 item, clinician rated scale, which includes 13 severity items and 3 diagnostic items. It has been shown to have a sensitivity of 92% and specificity of 95%, when a cut-off score of 17.75 is used to detect delirium (those with a score of > 17.75 have a positive screening test for delirium), however this is in a non-stroke population (Trzepacz PT 2001). The scale has also been found to be particularly useful in longitudinal studies where repeated measurements, including documentation of small changes in symptoms, are desirable (Trzepacz PT 2001). The DRS-R-98 was initially designed to evaluate the breadth and severity of delirium, and unlike other delirium rating scales (such as the CAM) was not based on any particular diagnostic criteria. In this study information from the nursing and clinical notes, as well as information gathered from interviewing the participant, was used to complete all sections of the DRS-R-98. This included responses given in the MoCA, where relevant, for the sections assessing memory, orientation and visuospatial ability. There are some inherent problems with the DRS-R-98 scale, for example it does not contain any items that assess for abnormal level of arousal, which as previously discussed, is a key feature of delirium. Furthermore, the score for the DRS-R-98 is more likely to be confounded by underlying dementia, as the scale includes memory items, although a recent study on a population with a high dementia prevalence found that the DRS-R98

performed well compared with diagnosis based on DSM-IV and International Classification of Diseases 10th edition (ICD-10) criteria (Sepulveda E et al 2015). The DRS-R-98 is a linear scale, with each item scoring either 0-3 or 0-2 points. The points are then added together to give a final score, with a higher score indicating more severe delirium. This means that equal weighting is given to inattention and long-term memory problems, but as I have already described inattention is the core feature of delirium, whereas long-term memory impairment may be associated with dementia, rather than being a manifestation of delirium. Finally, as some items may be reliant on participant recollection (if features such as hallucinations are not documented in case notes or recalled by clinical staff) inaccuracies resulting in a lower score may be introduced. For these reasons, once again the DRS-R-98 was not used to diagnose delirium, but rather to help to inform the diagnosis, based on DSM-IV criteria and to assess specific features and how they changed over time.

4.4.4 Digit Span Forwards and Backwards (from the Wechsler Adult Intelligence Scale III).

Digit span consists of progressively lengthening strings of numbers, read aloud by the tester and repeated aloud by the subject. The numbers are repeated in the same order given by the tester for the Digit Span Forwards test, and are repeated in reverse order for the backwards strand of the test. The scale is a test of attention and concentration and hence performance will be hindered by delirium, and is also a test of working memory and executive function (Golden 2002). Digit span scores are influenced by prior cognitive ability (IQ) and are detrimentally affected by the presence of dementia (Tieges, Brown et al. 2014). The value of objective tests for attentional deficits (rather than subjective judgements made from an overall clinical impression) have been demonstrated in several studies, although there is no current consensus as to which test is best employed to measure attention in studies of delirium (Tieges, Brown et al. 2014). Ideally any such test should be simple from a cognitive testing point of view, but demanding in terms of testing attention. Digit Span is discussed in more detail as a cognitive test in section 6.3.3.5.

4.4.5 DEL-box Mark 2

The Edinburgh Delirium Test Box Mark 2 (DEL-box, figure 4.4) is a computerised battery powered device which has been produced to provide sustained visual attention

tasks, as an assessment for the presence of delirium, which do not rely on the subjective assessment of the participants behaviour (Brown LJE 2012). The test box comprises two large illuminating response buttons, which light in a random fashion. Each task requires the subject to count the total number of illuminations they see. The tasks increase in complexity, with increasingly complex distracting stimuli being added to progressive tasks. The testing protocol used in this study consisted of seven test series, of increasing complexity, with one point scored for each correct test series response (the maximum score therefore being seven). In a small study (n=58) the DEL-box was shown to have good accuracy at distinguishing delirium from dementia and at distinguishing delirium from cognitively normal controls (Brown LJE 2012). The DEL-box Mark 2 has not been tested before in stroke patients, and in stroke patients in particular, visual fields should be checked prior to commencing the test, to ensure that a visual field deficit doesn't confound the test. If a homonymous hemianopia (or other visual field defect which may have significantly affected the participant's ability to complete the test) was detected, the DEL-box testing section of the delirium assessment was abandoned.

Figure 4.4 The Edinburgh Delirium Test Box Mark 2

4.4.6 The Observational Scale of Level of Alertness (OSLA)

The OSLA is a tool developed in Edinburgh, with the aim of detecting the bidirectional spectrum of change in alertness seen in delirium (Tieges 2013). This was developed as existing alertness scales had been found not to provide sufficient fine grained detail to detect subtle changes in arousal within the same participant and between participants. The scale has five anchored items which assess eye opening, eye contact, posture, movement and communication. A score of 4 or more (out of a possible 19) is taken to be abnormal. As this tool has only previously been used in exploratory studies, it was decided to use it in conjunction with the more well-established RASS.

4.4.7 Chart Confusion Assessment Method (Chart CAM)

For those participants who were recruited after day 1 following stroke, a retrospective review of casenotes was undertaken from the time of admission until time of recruitment, using the Chart CAM (Inouye 2005) as a frame of reference. The Chart CAM is a validated method for identifying delirium using chart review, and was found to have overall agreement with the Confusion Assessment Method (CAM) of 82%, and an interrater reliability kappa score of 0.41(Inouye, Leo-Summers et al. 2005). The Chart CAM involves systematically searching casenotes for any evidence of acute mental status change. Although it showed reasonable agreement with the CAM in the initial validation study, it must be noted that this was performed in the United States of America, where casenotes are different to those typically found in the United Kingdom (UK). For example, the nursing notes may have more clinical details in the American notes than they would in the UK. However, the Chart CAM provides a validated framework for extracting clinical information about mental status from casenotes, and in the absence of a UK version, was adopted for this study. Nursing and medical notes were reviewed, looking for any evidence of possible delirium. In addition to the casenote review the participant's case was discussed with the clinical team, with specific reference to any concerns about acute changes in cognition, level of arousal and attention (and any other features that would be in keeping with a

diagnosis of delirium). Finally, if available, the participant's condition over the days following admission to hospital was discussed with an informant. Any acute changes in cognition, attention or arousal (not coma), determined by casenote review and either informant or clinician history, which met the criteria for delirium, as set out in DSM-IV, were taken to be delirious episodes.

4.5 Delirium Diagnosis

As previously stated, the diagnosis of delirium was made by me, using the information gathered from the screening tests described above, to inform the diagnosis. Each of the four criteria laid out in DSM-IV (section 1.2.5) were addressed in turn, using the screening tool most applicable to each section to inform the diagnosis. For example, The OSLA and RASS scores were used to assess whether there was an altered level of arousal (Criterion 1), with an abnormality in either of these scores allowing a positive diagnosis for Criterion 1. In this same systematic way each criterion was addressed, with the screening tests applicable being used to inform the diagnosis. Criterion 1 also requires the 'reduced ability to sustain or shift attention, and for this section the results of the pertinent parts of the CAM-ICU, Digit Span and DEL-box tests were utilised, with an abnormal score for attention in any one of these tests allowing a positive score for this section. The DRS-R-98, CAM-ICU, digit span and MoCA were all used to help inform the scoring for criterion 2 ('A change in cognition or the development of a perceptual disturbance that is not better accounted for by a pre-existing, established or evolving dementia,') and CAM-ICU, DRS-R98, Chart CAM and informant/clinician discussion were used to inform the diagnosis for criterion 3 ('The disturbance develops over a short period of time (usually hours to days) and tends to fluctuate during the course of the day.') Finally, the casenotes, laboratory results and my own clinical judgement were used to inform the diagnosis for criterion 4 ('There is evidence from the history, physical examination or laboratory finding that the disturbance is caused by the direct physiological consequences of a general medical condition.')

4.6 Cognitive tests

4.6.1 Pre-morbid Intelligence Quotient (IQ)

The National Adult Reading Test (NART)

The National Adult Reading Test (Nelson 1991) was used to assess pre-morbid cognitive ability or intelligence quotient (IQ). The test was taken at the first opportunity when the patient was well enough to complete it (and so not during an episode of delirium for example). This test requires subjects to read aloud a set of 50 words which are irregularly spelt and have non-obvious pronunciation, for example "ache" and "depot". The responses are individually scored as correct or incorrect according to their pronunciation and this score is used to derive a premorbid IQ estimate. This is based on the assumption that reading ability is independent of cognitive decline (due to pathological processes such as dementia) and is a strong predictor of intelligence in the normal healthy population. McGurn and colleagues demonstrated that the NART is a valid estimate of premorbid IQ in those with mild to moderate dementia and several studies have demonstrated that NART correlates highly with full-scale IQ tests in the normal healthy population (McGurn, Starr et al. 2004). The NART was included in the study, as pre-morbid IQ significantly affects performance on many cognitive tests and so needs to be adjusted for in any subsequent analysis (Lezak 2004). A small number of participants who had a severe stroke, deteriorated rapidly and died were never well enough to complete the NART.

4.6.2 Pre-morbid cognitive impairment

The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)

The IQCODE was used to assess for prior, undiagnosed, cognitive impairment. This consists of a sixteen point questionnaire which is completed by someone who knows the participant well (usually the next of kin). The questions relate to changes in various aspects of cognition over the preceding 10 years, with the informant having to rate whether the participant has improved, deteriorated or stayed the same over the 10

years. The IQCODE has been validated as a screening test for dementia, and indeed has been found to perform at least as well as the Mini Mental State Examination (MMSE) (Jorm 1995). The short form of this questionnaire was developed in 1994 and is the version used in this study. The short form of IQCODE has been shown to be more suitable for detecting early cognitive decline, which is the main goal of the test in this study (Jorm 1995).

4.6.3 Current cognition

Current cognition was tested using a battery of neuropsychological tests described below, as well as the Montreal Cognitive Assessment (MoCA).

Montreal Cognitive Assessment (MoCA)

At baseline, 1 month, 4 months, and 12 months cognition was assessed using the MoCA (Nasreddine 2005). This test was selected as it has been found to have superior sensitivity over the more commonly used Mini Mental State Examination (MMSE) for detecting mild cognitive impairment (MCI) and has also been shown to have greater sensitivity to deficits in executive function, attention and delayed recall in those with cerebrovascular disease (Pendlebury 2010). The Addenbrook's Cognitive Examination-Revised (Mioshi, Dawson et al. 2006) is an alternative test which is commonly used in clinical practice, however has the disadvantage of being a longer test than the MoCA and hence was felt to be too burdensome to use for those in the immediate aftermath of an acute stroke. The MoCA tests eight cognitive domains, namely visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation. Participants are given an extra point if they have had 12 years or less of formal education and the overall score is out of a maximum of 30 points, with a normal score being 26 or more out of 30 (Nasreddine 2005).

Battery of Neuropsychological Tests

The neuropsychological test battery was administered at the 1 month, 4 month and 12 month follow-up assessments. All tests were attempted with each participant, unless

the participant became too fatigued to complete them, or asked for testing to be curtailed.

1. Visuospatial memory

Visual Reproduction

This test is part of the Wechsler Memory Scale-III (WMS-III) (Wechsler 1997). The subject is shown a series of 5 geometric designs of increasing complexity. The subject is given ten seconds to look at the first design, the design is then covered and the subject is asked to draw the design from memory. This process is repeated for the remaining four designs and the subject is then instructed to "try to remember all of the designs as later I will ask you to draw them again from memory." After 30 minutes the participant is asked to draw the designs again. Once they have completed this they are finally asked to simply copy each design as accurately as possible, this time without any time restrictions and without the image being removed before they commence drawing. Visual Reproduction is a test of visuospatial memory, and performance on this test is known to decline with age and also with dementia (Lezak 2004)

2. Verbal Memory

Controlled Oral Word Association Test

The Controlled Oral Word Association Test was used as a test of verbal fluency. The first part of the test involves the examiner giving a letter of the alphabet out loud (for this study the letters F, A and S, were used), and instructing the subject to speak out loud as many words as possible beginning with that letter in a one minute period. Subjects are told that proper nouns and the same words with different suffixes are not allowed. The second part of the test involves subjects being asked to name as many animals as possible in a one minute period. The animal names can begin with any letter. This test has been shown to be sensitive to frontal executive dysfunction (Hodges 2007) and semantic memory impairment. Performance is dependent on

education and has been shown to decline with ageing and in dementia (Hodges 2007). In general, category fluency (animal names) is usually superior to letter fluency.

HVLT-R List Learning

The Neuropsychological Assessment Battery HVLT-R List Learning Test (Stern 2003) was used as a test of episodic memory. The test has previously been shown to be able to differentiate between cognitively normal older adults and those with mild cognitive impairment or dementia when appropriate cut-off scores are used (Gavett, Poon et al. 2009). The test involves subjects being asked to try to remember a list of 12 words read aloud to them. The list is read three times and after each reading, the subject is asked which words they can recall. There is then a 20 minute delay (during which other cognitive testing is undertaken) and subjects are then asked if they can recall any of the 12 words (delayed recall). Finally a longer list of words is read to the subject, some of which were on the original list and some which were not. The subject has to identify correctly which words were on the original list (forced choice recognition).

3. Attention and Processing

Digit Span

The digit span forwards tests the ability of subjects to repeat a string of digits verbatim, beginning with two digits and increasing up to a maximum of nine consecutive digits. The reverse digit span tests the ability of subjects to repeat a string of digits in reverse order, beginning with a string of two digits and increasing up to a maximum of eight digits. For example the examiner may say “nine, one, seven,” and the subject must respond “seven, one, nine.” The numbers are read by the examiner at a rate of one per second, without clustering (which aids recall). This is a measure of attentional processes, and is dependent upon working (or “short-term”) memory, which in turn is dependent upon frontal executive function. Reduction in digit span scores is a feature of impaired attention, as seen in delirium, moderate to severe dementia and also in focal left hemisphere lesions (Hodges 2007). Subjects with

aphasia will therefore often have a reduced digit span score. In general, those with mild cognitive impairment will have a normal digit span score (Hodges 2007).

Digit Symbol Substitution Test (DSST)

The DSST is a part of the WMS-III (Wechsler 1997) and is a test of attention and processing speed. Subjects are presented with rows of numbers with blank boxes beneath them and are required to insert the appropriate symbols in the blank boxes using a key given at the top of the page. The test score is the number of correct symbol substitutions made in 90 seconds. The DSST score shows decline with age (Salthouse 1992) and in cognitive impairment, although it has been shown to be very sensitive to cognitive changes at high levels of cognition, but less sensitive to cognitive changes at low levels of cognition (such as in severe dementia) (Proust-Lima, Amieva et al. 2007).

Stroop Test

These tests involve subjects being asked to call out the names of six rows of coloured dots as rapidly as possible (task one), with the time taken to complete this being recorded. Subjects are then asked to call out the colour of ink six rows of words are printed in (task two) rather than the word itself (see row 2 figure 4.5 below), and finally are asked to call out the colour of ink that six rows of *colour* words are written in (task three), again with the time being recorded (see row three, figure 4.5) below. The tests are based on the fact that it takes longer to call out the names of coloured dots than it would do to read aloud words, and that it takes even longer to name the colour of ink in which a colour word is printed, especially if the colour of the word and the name of the word are not the same (for example blue is printed in green ink) A marked slowing of response in the third task is interpreted as cognitive slowing which is thought to be due to poor processing speed and a failure of selective attention (Hodges 2007). A markedly prolonged time to complete the third task when compared with the first and second task has been shown to be associated with early-stage Alzheimer's Dementia, as well as a variety of other brain pathologies including epilepsy (Hodges 2007).

Task one (shortened version)

when hard and over

Task two (shortened version)

blue yellow red green

Task three (shortened version)

Figure 4.5 A shortened version of the Victoria Stroop test.

4.7 Other outcome measures

At each of the follow-up visits (1 month, 4 months and 12 months), additional information about functional outcome was gathered as described below.

Nottingham Extended Activities of Daily Living score

The Nottingham Extended Activities of Daily living score (Nouri and Lincoln 1987) (Nottingham Extended ADL score) is a self-report questionnaire about activities actually done in the last few weeks. The scale has 22 ADL activities, and each activity is assigned a score of 0 or 1 depending on the response given. Four possible response options are given for each activity: "not at all," and "with help," either of which score 0 points, and "on my own with difficulty," or "on my own," either of which score 1 point. The maximum score for the test is therefore 22 points. This scoring tool has been shown to be valid in subjects who have had a stroke (Lincoln and Gladman 1992) and also has good test-retest reliability in this group (Nouri and Lincoln 1987) Whilst using a summative score for this tool as an outcome measure has been criticised for not providing a sensitive measure of small changes in function (das Nair, Moreton et al. 2011), it does provide a useful way of measuring general functional outcome, and of measuring changes in this over time.

4.8 Salivary cortisol sampling

Saliva, for measurement of cortisol, was obtained on the day of recruitment at two time points (0930) and (1530), and again on each subsequent assessment day at the same times. For the follow-up visits (1 month, 4 months and 12 months) those participants who were judged to have an intact swallow mechanism and be cognitively intact enough to follow written instructions (at the time of hospital discharge) were sent a saliva swab and instructions for collection of the saliva in the post the week before the scheduled visit. They were asked to collect a saliva sample on waking on the morning of my visit and to store it in their fridge for me to collect in the afternoon. A second sample was then collected by me in the afternoon following cognitive testing (at around 1530). For those who were unable to collect their own saliva sample, two visits were made to the participants home, one in the morning and

one in the afternoon. Samples were collected using either Salimetrics Oral Swabs (for those with an intact swallowing mechanism, as judged by the clinical team looking after the participant) or Salimetrics Children's Swabs (for those with an impaired swallowing mechanism). These two methods of collection are equivalent with respect to cortisol levels, according to the manufacturer's documentation . The swabs are made of an inert polymer, and are small, soft and tubular (see figures 4.6 and 4.7). The Oral Swabs are placed in the mouth, under the tongue or in the side of the mouth, and the Children's Swabs are held in place in the participant's mouth, by the person collecting the swab, for 60-90 seconds. The device collects approximately 200µL of saliva. Participants were closely assisted to obtain the samples and the written guidance on safe and effective use of the swab was closely followed. Participants were asked to abstain from eating, drinking, smoking or cleaning their teeth in the 20 minutes prior to sampling, as per the guidelines for use of the swabs. Following collection, samples were placed in a sealed tube, centrifuged for 10 minutes at 3000rpm, at room temperature, and then stored at -80˚C.

Figure 4.5 Salimetrics Oral Swab

Figure 4.6 Salimetrics Children's Swab

4.9 Analysis of CT brain scans

All participants underwent admission CT brain scanning (within 24 hours of stroke onset) as part of their clinical care prior to recruitment to the study. Subjects were scanned in a Toshiba 64-slice or 128-slice scanner (the 64-slice scanner being replaced by a new 128-slice scanner during the study period). Brain scans were stored in the clinical Patient Archiving and Communication System (PACS). A standard method of extracting information on stroke lesion location, anterior and posterior white matter lesions (using the Van Swieten scale (van Swieten, Hijdra et al. 1990)) and global cerebral atrophy (Pasquini, Leys et al. 2007), which has been successfully used in a previous study of fatigue after stroke (Kutlubaev, Shenkin et al. 2013) and also in a study of thrombolytic treatment after stroke (Wardlaw JM, Bradey N et al. 2015), was used for this study (see appendix 6) . Following recruitment to the study, but prior to delirium and cognitive assessments, the baseline clinical CT brain scan was rated using the standardised proforma previously described. Subsequently the scans were rated, in batches of ten, blind to all other data, by an experienced neuroradiologist (Professor Andrew Farrall, AJF). Scans were read by both AJB and AJF so that an inter-rater reliability analysis could be performed. With the exception of the inter-rater reliability, all results presented in chapter 8 are based on the data extracted solely by AJF. Further details are presented in chapter 8.

4.10 Statistical analysis

All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 14 and 19, with the exception of the random effects modelling which was carried out by Dr Mike Allerhand using the R statistics package and mixed effects multiple regression modelling which was carried out by Dr Daniel Davis using the Stata 13.1 statistics package. Assistance with the power calculation was provided by Professor John Starr. Specific analysis are described in detail in each chapter.

4.11 Power calculation

Assuming a sample size of 120 participants, and a delirium incidence of 30-40 % (Gustafson Y 1993), the main analyses are comparisons of cortisol levels between patients with (~N=40) and without (~N=80) delirium. Setting alpha at p=0.05 we have >80% power to detect medium-sized (Cohen's d=0.5) differences for these analyses. For logistic regression to determine predictors of specific cognitive decrements, setting alpha at p=0.05 and assuming an inter-correlation between risk factors of 0.3, and that 40% of participants show cognitive decline (from the time of stroke), n=84 provides 80% power to detect an odds ratio of 2.0 and n=94 provides 80% power to detect an odds ratio of 1.9. Hence, an attrition rate between a quarter and a third over one year is tolerable.

4.12 Ethical approval

The study was approved by the Scotland A Research Ethics Committee (reference number 12/SS/0141) and by NHS Lothian Research and Development (reference number 2012/R/ME/04)

Chapter 5: Delirium and salivary cortisol levels.

5.1 Introduction

It has long been hypothesised that glucocorticoids play a role in cognitive ageing, and indeed that glucocorticoid excess may reduce the brain's capacity to withstand neuropathological insults (Sapolsky, Krey et al. 1986). The effects of glucocorticoids on the brain, and in particular on the hippocampus, have been described as a nonlinear 'inverted-U' dose response curve. For example, in the hippocampus mild to moderate stressors may enhance synaptic plasticity, but more severe and prolonged stressors may cause hippocampal dendritic atrophy, and eventually permanent loss of hippocampal neurones (Sapolsky, Krey et al. 1986).

As discussed in chapter 1 section 1.4, glucocorticoids (specifically cortisol in humans) have been hypothesised to play an important role in the pathogenesis of delirium (MacLullich, Ferguson et al. 2008). Physiological stressors such as surgery and acute illness, and indeed psychological stress, may precipitate delirium (Fong, Tulebaev et al. 2009). As discussed in detail in section 1.4.4.1, impaired negative feedback regulation of cortisol, as well as loss of the circadian rhythm, has been demonstrated in ageing and also in those with neurodegenerative disease (Ferrari, Cravello et al. 2001, MacLullich, Ferguson et al. 2008). Delirium has also been shown to be associated with an impaired negative feedback (evidenced by an abnormal dexamethasone suppression test) in elderly patients with respiratory tract infection (O'Keeffe and Devlin 1994).

There are only five previous studies of cortisol and delirium after stroke. In a small study (n=23), Fassbender and colleagues (Fassbender, Schmidt et al. 1994) found an early and large activation of the HPA axis in response to acute stroke, but did not find any significant difference in cortisol levels between those who did and those who did not develop delirium (termed "acute confusional state" in this study.) Gustafson and colleagues (Gustafson Y 1993) studied 83 patients with acute stroke and found that both basal and post dexamethasone suppression test plasma cortisol levels were significantly higher in those with delirium. Interestingly, this study reported that 43% of participants developed delirium. This is higher than the majority of other studies,

which have tended to report an incidence of around 25% (Shi, Presutti et al. 2012). Stroke severity was not reported using a score (such as the NIHSS) in this study, rather the severity of paresis was recorded as none, slight, extensive or total. Thirty-five percent had no paresis, and only 16% had total paresis, suggesting that the study population probably did not have more severe stroke as an explanation for the higher delirium incidence. One possibility is that the method by which delirium was diagnosed was less prescriptive than that used in other studies. The authors were not clear as to how often participants were assessed during the first week after stroke, and describe using interviews with staff, patients and relatives to inform the DSM criteria, but are not specific about whether this information was used in isolation to make a diagnosis, or if objective testing was also required to confirm a diagnosis. Olsson (Olsson 1990) studied 20 stroke patients and found that those with "disorientation" had higher urinary cortisol compared with those who were orientated. This study did not look to diagnose delirium specifically, and those with undiagnosed dementia, for example, may therefore have been included in the "disorientated" group. A subsequent study by the same author (Olsson, Marklund et al. 1992) included 16 participants with stroke, and found that those with an "acute confusional state" had significantly higher urinary cortisol following dexamethasone suppression test, when compared to those without acute confusional state. The major limitation of this study is the small sample size. The final, and largest (n=88) of the previous studies, performed by Marklund and colleagues (Marklund, Peltonen et al. 2004), found that high serum cortisol in the first week after stroke was associated with disorientation. The diagnosis of disorientation was made using a 3 point scale (of the authors' own devising), and factors such as level of consciousness and orientation to time and place were considered. A diagnosis of delirium based on DSM criteria was not a part of the methodology.

In this chapter data gathered from measurement of salivary cortisol are presented, and then the hypothesis that delirium after stroke is associated with high circulating levels of cortisol is examined.

5.2 Methodology

5.2.1 Delirium assessment and salivary sample collection:

Delirium was diagnosed in the cohort according to DSM-IV criteria, as outlined in chapter 4. Diurnal salivary cortisol samples were obtained from each participant at each assessment time point (days 3,5 and 7, day 14 and 28 and 4 months and 12 months), and this methodology, including how samples were collected is also outlined in chapter 4. The normal range for salivary cortisol levels in healthy volunteers has been found to be 10-27nmol/l at 8am and 2-4nmol/l at 8pm (Laudat, Cerdas et al. 1988). The 'normal range' of salivary cortisol levels in stroke patients is difficult to define, as it is dependent on stroke type, severity and intercurrent illness, amongst other factors (Barugh, Gray et al. 2014).

5.2.2 Salivary cortisol analysis:

Saliva was collected using Salivette Swabs, and Salivette Children's Swabs (figure 4.5 and 4.6 chapter 4) for those with impaired swallowing mechanism (Salimetrics, Newmarket, Suffolk, UK). Following collection the Salivettes were centrifuged for 10 minutes at 3000rpm, at room temperature, and then stored at -80˚C. Samples were transported to The Dresden LabService Gmbh (Tatzberg 47-49, 01307, Dresden, Germany) on dry ice, where salivary cortisol Enzyme-Linked Immunosorbent Assays (ELISA) were performed.

5.2.3 Statistical analysis:

Morning and afternoon cortisol levels from each time point were plotted on simple line graphs and morning to afternoon ratios (to explore cortisol variability throughout the day) were calculated and plotted. The data was then explored and histograms to identify the distribution of the data were plotted. Participants were then divided into two groups, those who developed delirium at any time point over the course of the study and those who did not, and descriptive statistical analysis of the two groups was performed. A Mann Whitney U test was performed to compare unadjusted cortisol levels between the two groups.

Bivariate analysis:

Spearman's correlations were used to explore the relationship between cortisol levels and baseline characteristics of participants, specifically; age, NIHSS score, APACHE II score on admission, IQCODE score and Charlson Comorbidities Index. Biserial correlations were used to explore the relationship between cortisol levels and sex (binary outcome, male or female). Spearman's correlations were used to investigate the relationship between cortisol levels and continuous measures of delirium taken on the same day as the cortisol samples (the DRS-R98 and the Del-box) and biserial correlations were used to investigate the relationship between cortisol and dichotomised (positive or negative) measures of delirium (CAM-ICU (taken on the same day as the cortisol samples) and delirium diagnosis at any time point during the 12 months). For those who developed delirium, Spearman's correlations were used to explore the relationship between cortisol levels on the day of delirium diagnosis and delirium severity (as measured by the DRS-R98) on the same day. Finally, biserial correlations were used to investigate the relationship between the peak morning and peak afternoon cortisol levels during the first 14 days after stroke (morning and afternoon samples taken on the same day) and diagnosis of delirium at any point throughout the study period and to explore the relationship between median cortisol in the first 14 days after stroke and delirium diagnosis.

Multivariate analysis:

Multicollinearity was tested using variance inflation factor statistics.
Binary logistic regression, using the enter method, was used to explore the relationship between delirium diagnosis at any time throughout the study (as a binary outcome, yes or no) and median salivary cortisol levels in the week after stroke (day 0-7). Models were constructed using median morning and median afternoon cortisol levels. The first week was chosen as cortisol levels were found to be high in the first week after stroke in the systematic review reported in chapter 2. A second set of models, using binary logistic regression were constructed using the peak cortisol levels (morning, afternoon and the ratio of morning to afternoon) during the first 14 days after stroke. The first 14 days was used for this analysis (rather than the first 7 days) in order to capture any later peaks. Important covariates selected a priori were

age, sex, NIHSS score, IQCODE, Charlson Comorbidities index and APACHE II score, thus the analysis was designed to estimate the effect of cortisol on delirium independent of stroke severity, acute illness severity, chronic illness burden and prior cognitive impairment.

Finally a generalised linear random effects model was fitted to investigate the effect of cortisol (morning, afternoon and ratio of morning: afternoon) over time on presence or absence of delirium. This analysis was performed by Dr Mike Allerhand, Centre for Cognitive Ageing and Cognitive Epidemiology statistician, University of Edinburgh. The outcome variable was presence of delirium at any point during the study (yes or no), and the predictor variable was salivary cortisol level. The model was specified with binomial errors and a logit link function, which is appropriate for predicting the odds of group membership using a binary outcome variable (in this instance delirium, yes or no). The analysis was conducted using the GLMER function in the LME4 R statistics package. As these were both hypothesis-driven but also exploratory analyses, data for each time point are presented.

5.3 Results

95 participants were recruited, and of these 26 developed delirium at some point throughout the 12 month study period. Participant baseline information is summarised in table 5.1. Those who developed delirium were older, more likely to be female, less likely to be independent in activities of daily living (ADLs) prior to stroke, more likely to have a pre-existing cognitive impairment (evidenced by IQCODE score), more likely to have had a more severe stroke (as measured by the NIHSS score) and more likely to have had a TACS stroke, when compared with those who did not develop delirium. Table 5.2 summarises the timing of onset, duration and severity of delirium for each of the 26 participants that developed delirium during the study period. The median day of delirium onset was day 4 (IQR day 2 - day 6), the median duration was 2 days (IQR 1 day - 6 days). The median delirium severity score, at the time of delirium diagnosis, (measured by the DRS-R98) was 22 (IQR 16-26).

Table 5.1 Baseline characteristics for the whole cohort, and for those who developed delirium during the study period and those who did not.

Characteristic	Whole cohort (n=95) Median (interquartile range)	Delirium (n=26) Median (interquartile range)	No delirium (n=69) Median (interquartile range)	p
Age	77 years (71-84)	83.5years (79-85.3)	74 years (68.5-82)	<0.001 [a]
Male sex	N=56 (59%)	N=10 (38%)	N=46 (67%)	0.013[b]
Time in formal education	11 years (11-13)	11 years (10-12)	11 years (11-14)	0.036[a]
Independent in ADL's pre-stroke	N=81 (85%)	N=17 (65%)	N=64 (67%)	0.006[b]
IQCODE score	3.25 (3-3.69)	3.56 (3.19-4.6)	3.19 (3.0-3.7)	0.022 [a]
NART score	35(29.25-41)	34.5 (25.25-40.25)	36 (29.75-41.25)	0.356 [a]
NIHSS score	5 (4-8)	8.5 (5-12.75)	5 (3-7)	0.009 [a]
APACHE II score	8 (6-10)	9 (7.75-12.25)	7 (6-10)	0.350 [a]
Charlson co-morbidity index	2 (1-4)	2 (1.75-4)	2 (1-4)	0.572 [a]
Stroke type (OCSP)	TACS 15 PACS 35 LACS 33 POCS 12	TACS 12 PACS 8 LACS 5 POCS 1	TACS 3 PACS 27 LACS 28 POCS 11	<0.001[b]

[a] Mann-Whitney U test
[b] Pearson chi-square

Table 5.2 Timing and duration of delirium for the 26 participants who developed delirium during the study, with maximal delirium severity score at the time of delirium diagnosis.

Participant	Day delirium first diagnosed	Day delirium resolved	Duration of delirium (days)	Delirium severity (DRS-R98)
1	12	21	9	19
2	5	12	7	25
3	1	12	12	27
4	1	Participant died	-	24
5	2	Participant died	-	33
6	5	7	2	22
7	14	15	1	11
8	1	3	2	4
9	4	8	4	22
10	3	5	2	30
11	3	Participant died	-	30
12	365	-	-	22
13	1	5	4	7
14	6	112	-	24
15	5	7	2	23
16	5	7	2	17
17	28	112	-	8
18	5	112	-	22
19	4	112	-	35
20	4	5	1	18
21	1	5	4	17
22	5	Participant died	-	28
23	1	5	4	12
24	28	112	-	22
25	3	5	2	6
26	6	14	8	23

5.3.1 Cortisol levels: Descriptive statistics

It was not possible to obtain diurnal cortisol results for all 95 participants at each assessment. The reasons for missing samples were that: the participant declined, was too unwell to take the test or forgot to take the sample prior to a home visit. Descriptive data for cortisol at each time point, including number of samples available, are given in table 5.3. As expected, morning cortisol levels were higher than the afternoon levels at each time point. Histograms for morning and afternoon cortisol levels taken on day 5 are shown in figures 5.1 and 5.2 (histograms for other time points can be found in appendix 7) and were chosen as being representative of all of the histograms from all of the time points cortisol was measured at. These show that cortisol levels are positively skewed, although this effect is lessened when extreme outliers are removed. However, as the data cannot be said to be normally distributed, non-parametric statistical analysis was used. Scatterplots (appendix 8) revealed two extreme outliers (participant 2 and participant 71, neither of whom developed delirium). All bivariate and multivariate analysis were run with and then without the outliers. No significant differences in results were found between these two sets of analysis, and so data is presented with the outliers excluded.

Table 5.3 Cortisol levels: descriptive data.

Timing of cortisol sample	n	Median (nmol/L)	IQR (nmol/L)
9am cortisol day 3	48	23.74	17.33-30.87
3.30pm cortisol day 3	45	14.13	9.15-19.42
Ratio am: pm cortisol day 3	43	1.71	1.22-2.99
9am cortisol day 5	70	24.43	18.70-32.52
3.30pm cortisol day 5	66	14.10	10.37-19.60
Ratio am: pm cortisol day 5	64	1.68	1.19-2.48
9am cortisol day 7	52	21.76	16.80-30.27
3.30pm cortisol day 7	47	12.43	10.38-16.89
Ratio am: pm cortisol day 7	45	1.52	1.32-2.41
9am cortisol day 14	37	22.69	16.03-29.04
3.30pm cortisol day 14	30	12.81	9.50-17.00
Ratio am: pm cortisol day 14	27	1.76	1.13-2.28
9am cortisol day 28	77	22.80	17.73-34.73
3.30pm cortisol day 28	74	12.80	9.25-17.01
Ratio am: pm cortisol day 28	65	1.92	1.41-2.85
9am cortisol 4 months	65	23.07	16.26-30.55
3.30pm cortisol 4 months	74	12.62	8.65-16.49
Ratio am: pm cortisol 4 months	59	1.83	1.20-2.60
9am cortisol 12 months	68	24.50	17.28-21.31
3.30pm cortisol 12 months	67	11.70	6.53-22.15
Ratio am: pm cortisol 12 months	63	2.00	1.17-3.38

Figure 5.1 Histogram for 9.30 am day 5 cortisol

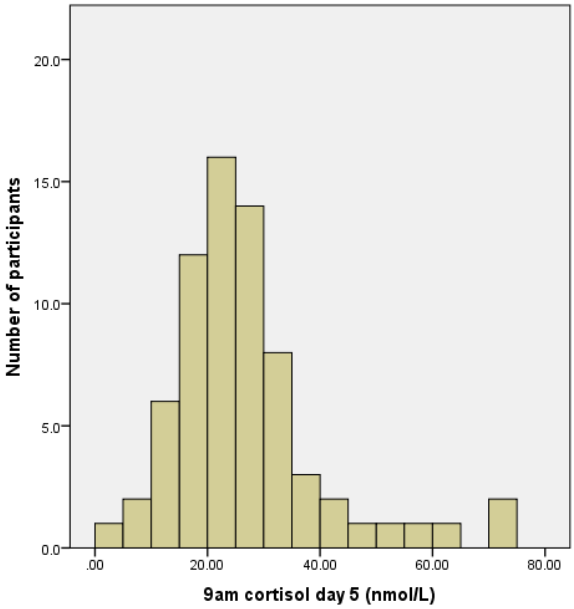
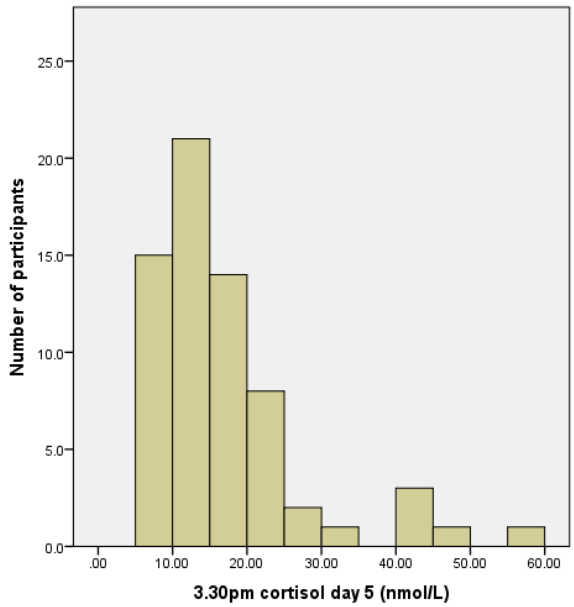


Figure 5.2 Histogram for 3.30 pm day 5 cortisol



5.3.2 Cortisol levels in those with and without delirium

Descriptive data for cortisol levels at each time point, split into those who developed delirium at any point throughout the 1 year study period, and those who did not are shown in table 5.4. Both groups showed diurnal variation in cortisol levels, with higher levels at 9.30 am compared with 3.30pm. Morning and afternoon cortisol levels in those who did and those who did not develop delirium at each time point are also illustrated in box and whisker plots in figure 5.3 (a-j) Line graphs illustrating morning and afternoon cortisol levels and variability in cortisol (as indexed by morning to afternoon cortisol ratio) are shown in figure 5.4 (a-c).

5.3.3 Bivariate analysis

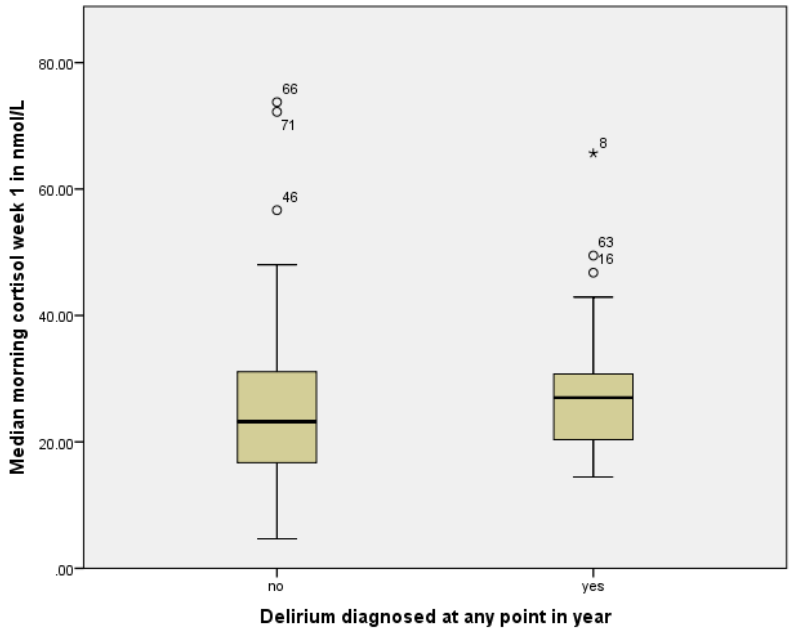
Cortisol levels were significantly higher in the delirium group, when compared with the non-delirium group, at 9.30 am on day 3 (median 29.76 nmol/L versus median 22.58nmol/L, Mann-Whitney U test p=0.04), 3.30pm on day 5 (median 18.18nmol/L versus median 13.45nmol/L, Mann-Whitney U test p=0.04), 9.30 am and 3.30pm on day 14 (am median 32.18nmol/L versus 18.35nmol/L, Mann-Whitney U test p=0.01 and pm median 15.94nmol/L versus 11.92 nmol/L, Mann-Whitney U test p=0.02), 3.30pm on day 28 (pm median 16.41nmol/l versus 12.19nmol/L, Mann-Whitney U test p=0.03) and at 9.30 am at 4 months (median cortisol 29.81nmol/L versus 22.43 nmol/L, Mann-Whitney U test p= 0.04). There were no significant differences between cortisol levels in the two groups at 12 months post stroke.

Table 5.4 Cortisol levels: descriptive data comparing those who did and those who did not develop delirium (◊ Mann-Whitney U test)

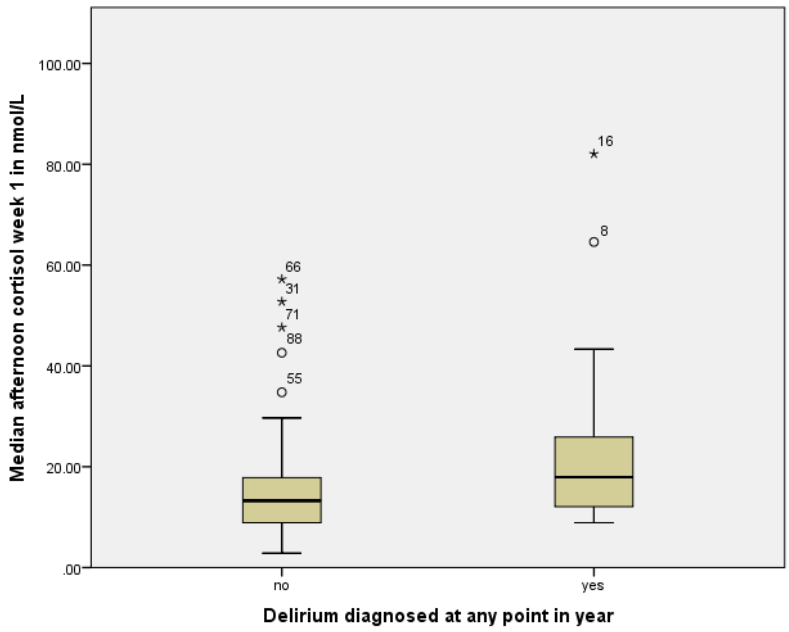
Cortisol sample (nmol/L)	**No delirium (n=69)**			**Delirium (n=26)**			**p◊**
	n	Median	IQR	n	Median	IQR	
9.30 am cortisol day 3	39	22.58	14.16-30.41	9	29.76	25.10-38.89	0.04
3.30pm cortisol day 3	37	13.15	8.53-18.08	8	20.14	10.83-54.81	0.05
Ratio am:pm cortisol day 3	36	1.72	1.26-2.28	7	1.41	1.02-3.03	0.55
9.30 am cortisol day 5	49	24.33	18.5-30.42	21	24.53	18.81-33.20	0.74
3.30pm cortisol day 5	48	13.45	9.3-17.57	18	18.18	13.11-20.95	0.04
Ratio am:pm cortisol day 5	46	1.87	1.28-2.58	18	1.44	0.95-1.75	0.02
9.30 am cortisol day 7	35	21.72	16.75-33.06	17	23.12	16.07-27.66	0.66
3.30pm cortisol day 7	32	11.58	9.25-16.73	15	14.48	10.95-16.96	0.58
Ratio am:pm cortisol day 7	31	1.51	1.16-2.46	14	1.57	1.40-2.26	0.93
9.30 am cortisol day 14	26	18.35	15.17-24.51	11	32.18	17.15-43.17	0.01
3.30pm cortisol day 14	19	11.92	9.32-15.86	11	15.94	12.80-20.61	0.02
Ratio am:pm cortisol day 14	18	1.65	1.24-2.38	9	1.77	1.02-2.21	0.94
9.30 am cortisol day 28	59	21.92	17.88-30.82	18	31.25	16.48-39.06	0.22
3.30pm cortisol day 28	60	12.19	8.95-15.66	14	16.41	11.07-22.54	0.03
Ratio am:pm cortisol day 28	54	1.93	1.4-2.93	11	1.66	1.41-2.00	0.48
9.30 am cortisol 4 months	50	22.43	15.65-27.03	15	29.81	18.66-34.15	0.04
3.30pm cortisol 4 months	60	12.52	7.9-15.72	14	14.46	8.93-19.11	0.28
Ratio am:pm cortisol 4 months	48	1.83	1.16-2.66	11	1.98	1.41-2.21	0.85
9.30 am cortisol 12 months	53	24.61	16.64-37.6	15	23.57	18.2-36.5	0.91
3.30pm cortisol 12 months	53	11.33	6.22-22.17	14	13.94	7.94-22.56	0.42
Ratio am:pm cortisol 12 months	50	2.09	1.18-3.4	13	1.9	1.33-3.26	0.97

Figure 5.3 (a-j) Box and whisker plots of cortisol levels (morning and afternoon) for those who did and those who did not develop delirium

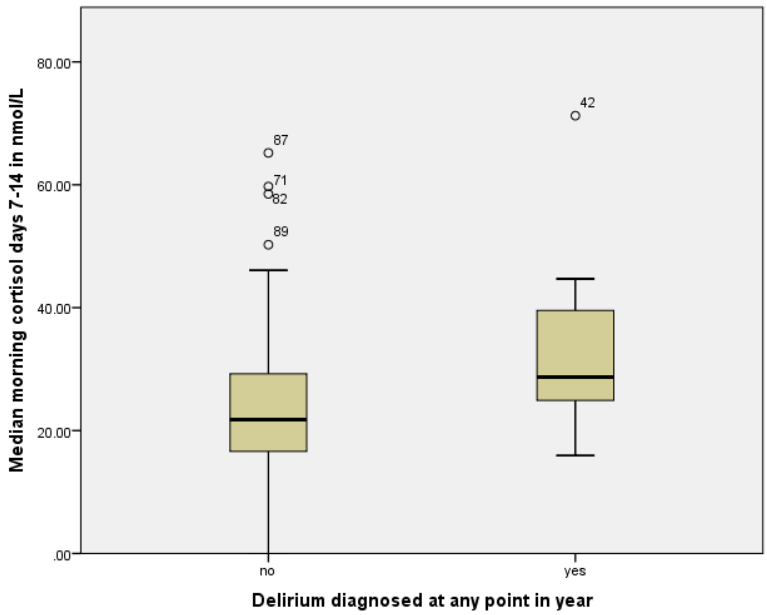
a. Median morning cortisol levels during week 1 for those who did and those who did not develop delirium



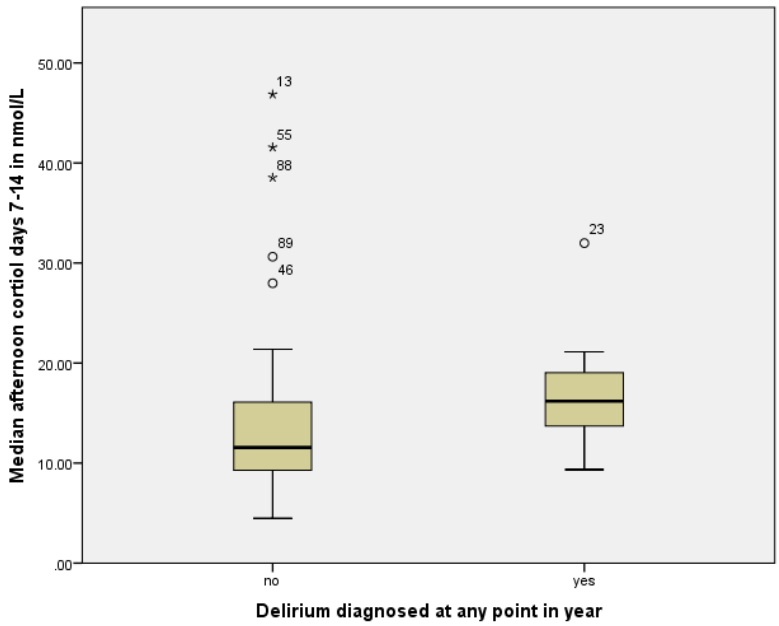
b. Median afternoon cortisol levels during week 1 for those who did and those who did not develop delirium



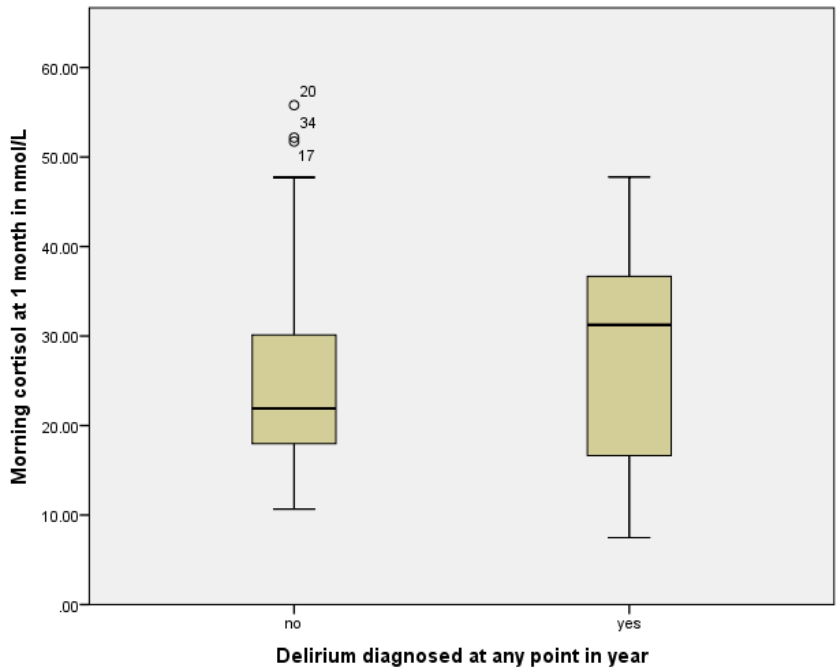
c. Median morning cortisol levels day 7-14 for those who did and those who did not develop delirium



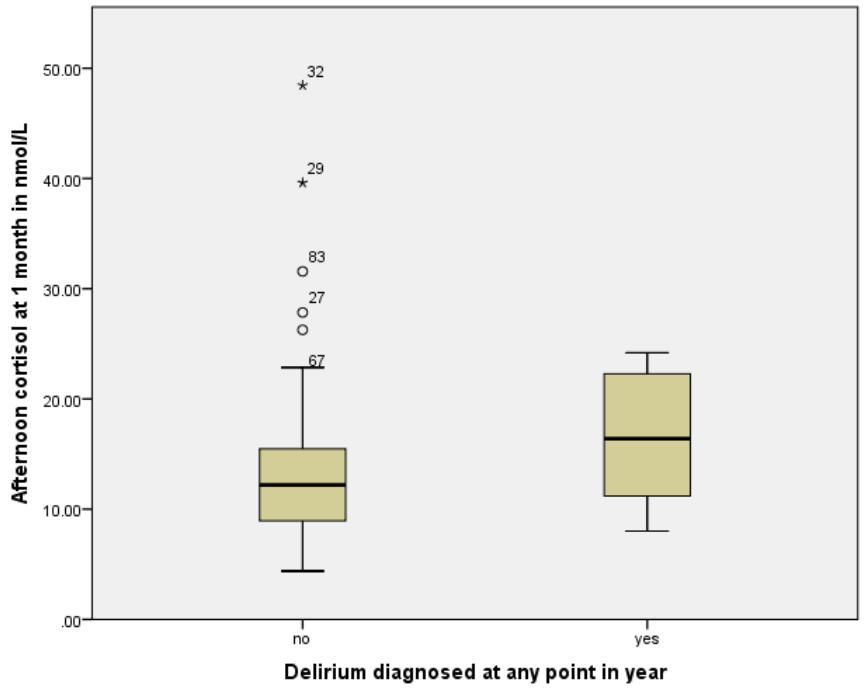
d. Median afternoon cortisol levels day 7-14 for those who did and those who did not develop delirium



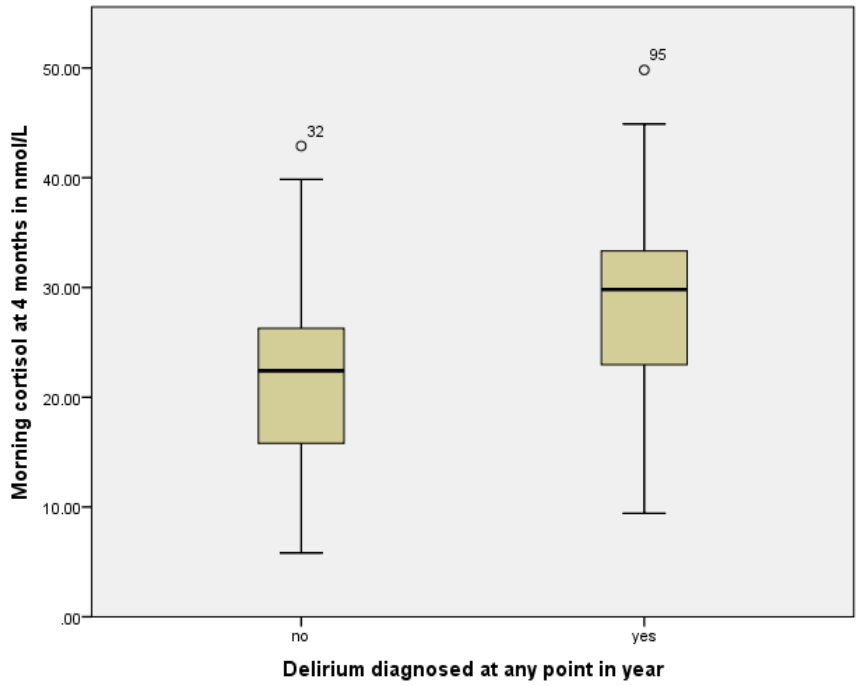
e. Median morning cortisol levels at 1 month for those who did and those who did not develop delirium



f. Median afternoon cortisol levels at 1 month for those who did and those who did not develop delirium



g. Median morning cortisol levels at 4 months for those who did and those who did not develop delirium



h. Median afternoon cortisol levels at 4 months for those who did and those who did not develop delirium

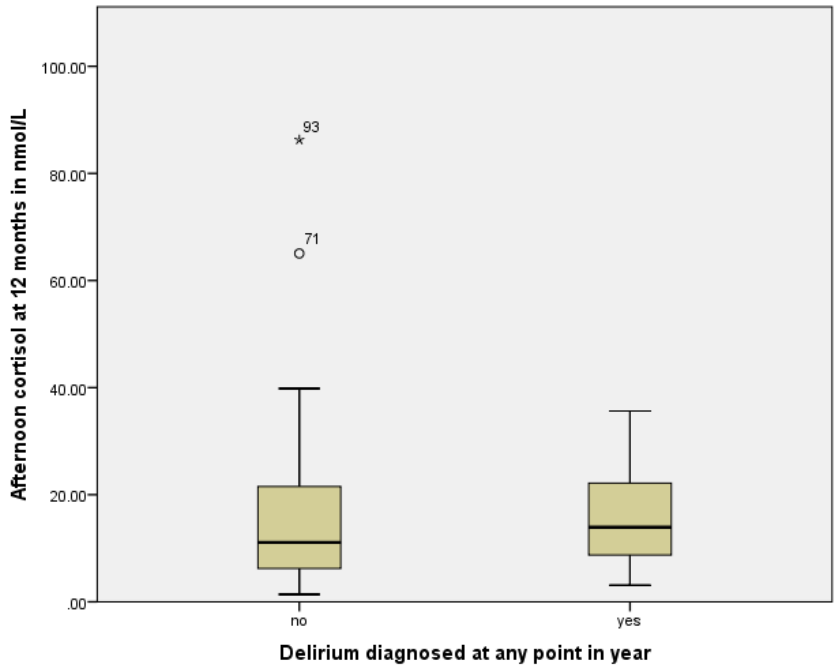
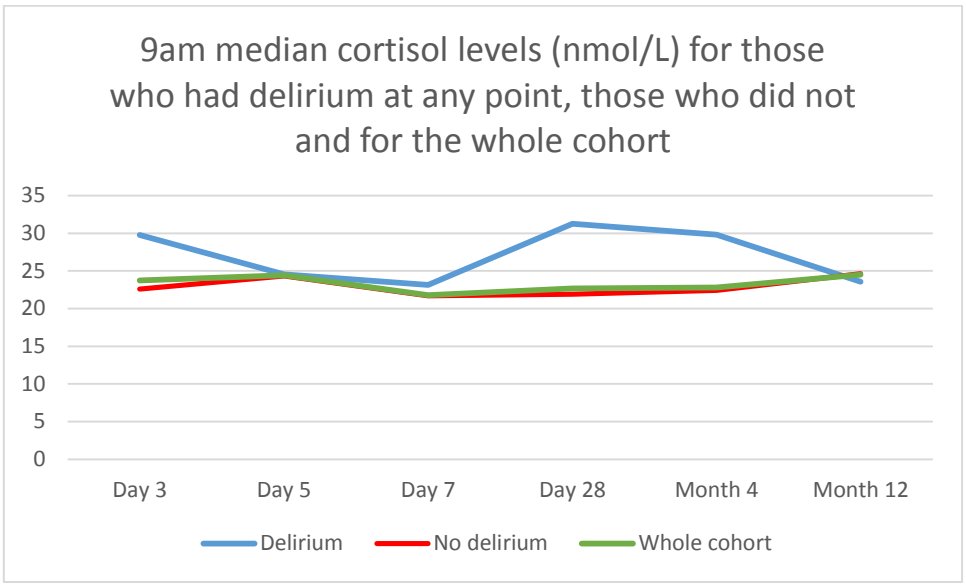
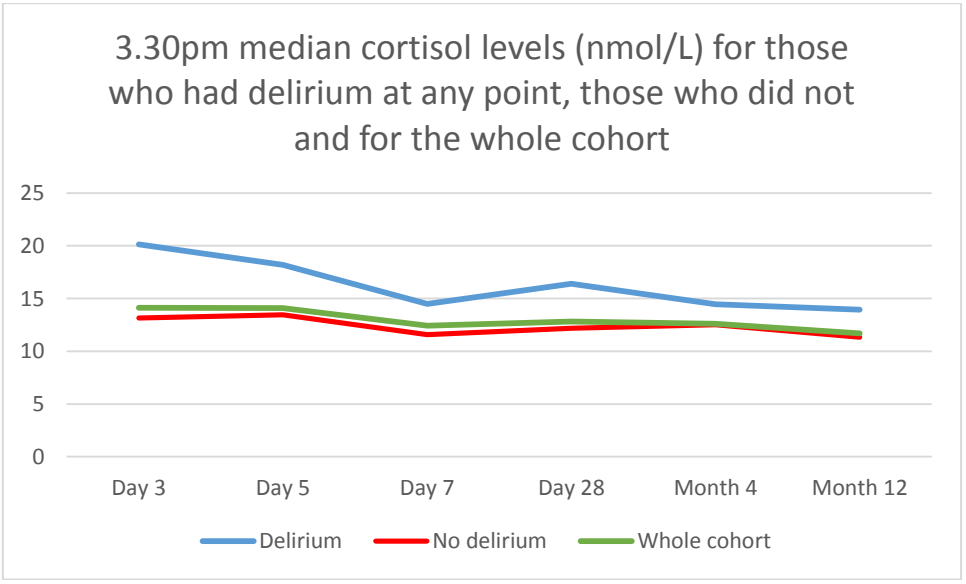


Figure 5.4 Line graphs of 9.30 am (a), 3.30 pm (b) and ratio of morning to afternoon (c) median salivary cortisol levels in those who did (blue line) and did not (red line) develop delirium and for the whole cohort (green line)

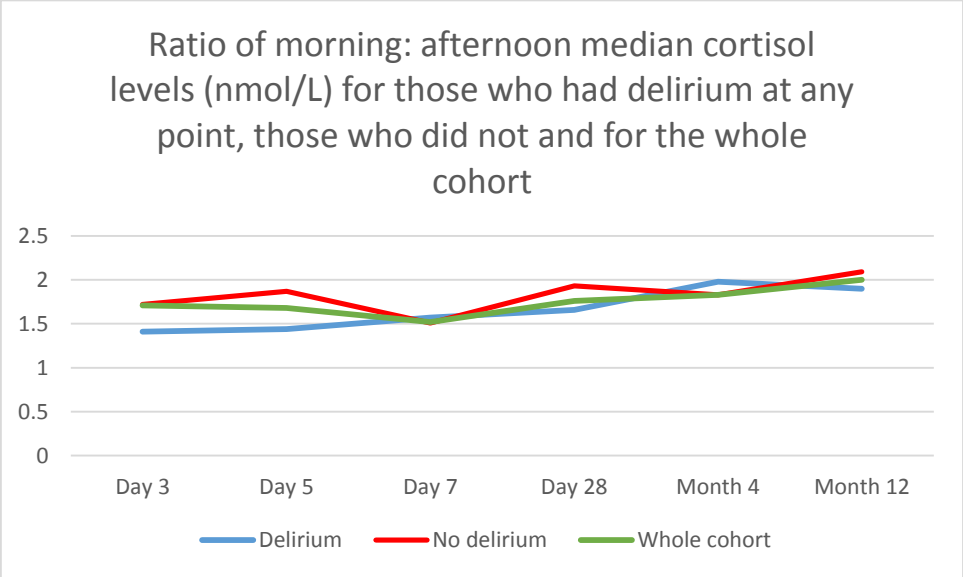
a.



b.



c



5.3.4 Correlations between cortisol levels and baseline characteristics

Correlations between cortisol levels and baseline characteristics are shown in table 5.5. This shows that 9.30 am cortisol taken on day 3 significantly correlated with age, admission NIHSS score and admission APACHE II score. 3.30pm cortisol on day 3, day 14 and day 28 significantly correlated with admission APACHE II score, as did 9am cortisol at 12 months. 3.30pm cortisol on day 3 and day 28 significantly correlated with admission NIHSS score. 3.30 pm cortisol on day 5 significantly correlated with participants' age as did the ratio of morning to afternoon cortisol at 12 months. Finally 3.30 pm cortisol at 4 months significantly correlated with the Charlson Comorbidities Index.

Table 5.5 Correlations between cortisol levels baseline characteristics

Cortisol sample (nmol/L)	Age (years)	NIHSS score on admission	APACHE II score on admission	IQCODE score	Charlson Comorbidities Index score	Sex
9am cortisol day 3	0.357 (p=0.013)	0.425(p=0.003)	0.503 (p=<0.001)	0.041 (p=0.798)	0.143 (p=0.331)	0.187 (p=0.203)
3.30pm cortisol day 3	0.168 (p=0.269)	0.381 (p=0.010)	0.362 (p=0.015)	-0.108 (p=0.505)	-0.054 (p=0.725)	0.230 (p=0.129)
Ratio am:pm cortisol day 3	-0.002 (p=0.988)	0.009 (p=0.955)	0.159 (p=0.308)	0.004 (p=0.983)	0.159 (p=0.309)	0.203 (p=0.191)
9am cortisol day 5	0.297 (p=0.012)	0.139 (p=0.251)	0.232 (p=0.054)	-0.066 (p=0.609)	0.012 (p=0.920)	-0.085 (p=0.486)
3.30pm cortisol day 5	0.392 (p=0.001)	0.075 (p=0.547)	0.237 (p=0.055)	0.085 (p=0.513)	0.02 (p=0.874)	-0.098 (p=0.432)
Ratio am:pm cortisol day 5	-0.087 (p=0.493)	-0.091 (p=0.477)	-0.091 (p=0.472)	-0.017 (p=0.898)	0.046 (p=0.720)	0.062 (p=0.626)
9am cortisol day 7	-0.043 (p=0.762)	-0.170 (p=0.229)	-0.024 (p=0.868)	0.024 (p=0.878)	-0.174 (p=0.216)	-0.036 (p=0.797)
3.30pm cortisol day 7	0.139 (p=0.351)	-0.050 (p=0.737)	0.243 (p=0.099)	-0.150 (p=0.357)	-0.104 (p=0.486)	-0.248 (p=0.093)
Ratio am:pm cortisol day 7	-0.144 (p=0.346)	-0.118 (p=0.439)	-0.164 (p=0.281)	0.082 (p=0.623)	-0.197 (p=0.195)	0.149 (p=0.329)
9am cortisol day 14	0.338 (p=0.041)	-0.052 (p=0.459)	0.303 (p=0.068)	0.134 (p=0.459)	0.072 (p=0.670)	0.338 (p=0.041)
3.30pm cortisol day 14	0.537 (p=0.002)	0.225 (p=0.232)	0.419 (p=0.021)	0.173 (p=0.379)	0.084 (p=0.658)	0.064 (p=0.737)
Ratio am:pm cortisol day 14	-0.006 (p=0.978)	-0.193 (p=0.336)	-0.012 (p=0.954)	0.147 (p=0.482)	-0.144 (p=0.473)	0.218 (p=0.275)

9am cortisol day 28	0.049 (p=0.672)	0.046 (p=0.691)	0.038 (p=0.740)	-0.010 (p=0.936)	-0.074 (p=0.521)	-0.001 (p=0.996)
3.30pm cortisol day 28	0.390 (p=0.001)	0.233 (p=0.045)	0.357 (p=0.002)	-0.048 (p=0.698)	0.273 (p=0.019)	-0.182 (p=0.120)
Ratio am:pm cortisol day28	-0.203 (p=0.105)	-0.213 (p=0.089)	-0.220 (p=0.078)	0.008 (p=0.950)	-0.219 (p=0.08)	-0.11 (p=0.932)
9am cortisol 4 months	0.168 (p=0.181)	0.122 (p=0.333)	-0.078 (p=0.536)	0.154 (p=0.236)	-0.045 (p=0.721)	-0.123 (p=0.328)
3.30pm cortisol 4 months	0.072 (p=0.541)	0.109 (p=0.356)	0.113 (p=0.336)	0.130 (p=0.291)	0.307 (p=0.008)	-0.140 (p=0.234)
Ratio am:pm cortisol 4 months	0.033 (p=0.805)	-0.070 (p=0.600)	-0.165 (p=0.212)	-0.011 (p=0.937)	-0.251 (p=0.055)	-0.112 (p=0.400)
9am cortisol 12 months	-0.250 (p=0.04)	0.065 (p=0.598)	-0.252 (p=0.038)	-0.053 (p=0.675)	0.053 (p=0.669)	-0.094 (p=0.445)
3.30pm cortisol 12 months	0.094 (p=0.447)	0.068 (p=0.587)	-0.051 (p=0.681)	0.103 (p=0.422)	0.207 (p=0.093)	-0.105 (p=0.399)
Ratio am:pm cortisol 12 months	-0.169 (p=0.185)	-0.012 (p=0.925)	-0.058 (p=0.651)	-0.138 (p=0.297)	-0.129 (p=0.313)	-0.308 (p=0.014)

5.3.5 Correlations between cortisol levels and measures of delirium

Table 5.6 shows correlations between cortisol levels at each time point and measures of delirium taken at the same time point. Afternoon cortisol levels significantly correlated with the three delirium rating scores (DRS-R98, DEL-box and CAM-ICU) on day 3 and day 5 after stroke and correlated with one score (DRS-R98) on day 14 and with two (DEL-box and CAM-ICU) on day 28. Morning cortisol levels significantly correlated with the three delirium rating scores on day 14 and with the CAM-ICU alone on day 28. The ratio of morning to afternoon cortisol correlated significantly with the CAM-ICU on day 3, with all three delirium rating scores on day 5 and with the DRS-R98 on day 28.

There were no significant correlations between any of the cortisol measurements taken at 4 months and 12 months and measures of delirium.

Table 5.6 Correlations between cortisol levels at each time point and measures of delirium taken at the same time point.

Cortisol sample (nmol/L)	DRS-R98 (Spearman's rho)	Del-box (Spearman's rho)	CAM-ICU (biserial correlation)
9am cortisol day 3	0.226(p=0.063)	-0.150(p=0.182)	0.187(p=0.104)
3.30pm cortisol day 3	0.322(p=0.015)	0.412(p=0.005)	-0.66(p<0.001)
Ratio am:pm cortisol day 3	-0.182(p=0.121)	0.167(p=0.161)	-0.256(p=0.049)
9am cortisol day 5	0.07(p=0.29)	0.021(p=0.43)	-0.010(p=0.47)
3.30pm cortisol day 5	0.30(p=0.007)	0.259(p=0.019)	-0.239(p=0.03)
Ratio am:pm cortisol day 5	0.273(p=0.01)	0.336(p=0.004)	0.34(p=0.003)
9am cortisol day 7	0.067(p=0.318)	0.029(p=0.424)	-0.176(p=0.106)
3.30pm cortisol day 7	0.126(p=0.200)	-0.344(p=0.012)	-0.006(p=0.483)
Ratio am:pm cortisol day 7	0.071(p=0.321)	0.260(p=0.051)	0.132(p=0.194)
9am cortisol day 14	0.343(p=0.02)	-0.394(0.012)	0.453(p=0.003)
3.30pm cortisol day 14	0.369(p=0.025)	-0.144(0.233)	0.131(0.249)
Ratio am:pm cortisol day 14	0.171(p=0.201)	-0.315(p=0.062)	0.195(p=0.170)
9am cortisol day 28	-0.135(p=0.121)	-0.175(p=0.076)	0.234(p=0.022)
3.30pm cortisol day 28	0.167(p=0.077)	-0.37(p=0.001)	0.208(p=0.041)
Ratio am:pm cortisol day28	0.330(p=0.004)	0.209(p=0.059)	-0.104(p=0.210)
9am cortisol 4 months	0.090(p=0.243)	0.080(p=0.267)	0.180(p=0.155)
3.30pm cortisol 4 months	0.100(p=0.204)	0.092(p=0.220)	NA
Ratio am:pm cortisol 4 months	-0.072(p=0.299)	0.068(p=0.308)	NA
9am cortisol 12 months	0.008(p=0.008)	-0.016(p=0.449)	0.050(p=0.345)
3.30pm cortisol 12 months	0.205(p=0.050)	-0.086(p=0.246)	0.168(p=0.089)
Ratio am:pm cortisol 12 months	-0.186(p=0.074)	0.047(p=0.358)	-0.076(0.279)

NA- Not applicable as CAM-ICU negative for all subjects

5.3.6 Correlations between median cortisol levels (day 0-7) and delirium diagnosis

Table 5.7 shows the Biserial correlations between median 9am and median 3.30pm cortisol levels in the first week after stroke and diagnosis of delirium at any time point during the study. This shows that there is a significant correlation between median afternoon cortisol levels and delirium diagnosis, but no significant correlation was found between median 9am cortisol and delirium.

Table 5.7 Correlations between median cortisol levels (day 0-14) and delirium diagnosis

Median cortisol level (nmol/L) (day 0-7)	**Delirium diagnosis at any point in study (Biserial correlation)**
Median 9am cortisol	0.170 (p=0.1)
Median 3.30pm cortisol	0.226 (p=0.03)

5.3.7 Correlations between cortisol levels at the time of delirium diagnosis and delirium severity

The median day that delirium was diagnosed was day 5 after stroke (IQR day 4-day 7). Table 5.8 shows the Spearman's correlations between cortisol levels on the day of delirium diagnosis and delirium severity on that same day (DRS-R98). This shows that both 3.30pm cortisol and the morning to afternoon ratio are significantly correlated with the DRS-R98 score (as a measure of delirium severity).

Table 5.8 Correlations between cortisol levels at the time of delirium diagnosis and delirium severity (rated by the DRS-R98)

Cortisol sample (nmol/L)	DRS-R98 (Spearman's rho)
9am cortisol on day delirium diagnosed	0.225(p=0.157)
3.30pm cortisol on day delirium diagnosed	0.398(p=0.05)
Ratio of morning: afternoon cortisol on day delirium diagnosed	-0.485(p=0.021)

5.3.8 Correlations between peak cortisol levels in the first 14 days after stroke and delirium diagnosis

The median day that peak levels of cortisol were obtained was day 5 after stroke onset (IQR 3-7). Table 5.9 shows the Biserial correlations between delirium diagnosis at any point during the study and peak cortisol during the first 14 days. This shows that peak 3.30pm cortisol is significantly correlated with delirium diagnosis, but peak morning levels and the peak ratio are not.

Table 5.9 Correlations between peak cortisol levels in the first 14 days after stroke and delirium diagnosis

Cortisol sample (nmol/L)	Delirium diagnosed at any point in study (Biserial correlation)
Peak 9am cortisol	0.048(p=0.652)
Peak 3.30pm cortisol	0.302(p=0.004)
Ratio of peak morning: afternoon cortisol	-0.174(p=0.107)

5.3.9 Multivariate analysis:

The assumptions for logistic regression including linearity and independence of errors were tested and met. Multicollinearity was tested using variance inflation factor statistics. The models are corrected for all of the variables presented.

Median 9.30 am cortisol (days 0-7):
Complete data were available for 86 participants and the model accounted for between 33% and 48 % of the variance in delirium status. Table 5.10 shows the odds ratio and 95% confidence intervals (CI) for each of the predictor variables. This shows that only age and NIHSS score on admission were independent predictors of delirium incidence, with mean 9am cortisol (days 0-7) approaching statistical significance (OR 0.95, p=0.08)

Median 3.30pm cortisol (days 0-7):
Complete data were available for 83 participants and the model accounted for between 27% and 40% of the variance in delirium status. Table 5.11 shows the odds ratio and 95% confidence intervals for each of the predictor variables. This shows that only age and NIHSS on admission were independent predictors of delirium incidence.

Ratio of median morning to afternoon cortisol (days 0-7):
Complete data were available for 83 participants and the model accounted for between 30% and 45% of the variance in delirium status. Table 5.12 shows the odds ratio and 95% confidence intervals for each of the predictor variables. This shows that only NIHSS score on admission was an independent predictor of delirium incidence.

Peak 9.30 am cortisol (days 0-14)
Complete data were available for 84 participants and the model accounted for between 37% and 55% of the variance in delirium status. Table 5.13 shows the odds ratio and 95% confidence intervals for each of the predictor variables. This shows that only age, NIHSS score on admission and IQCODE score were independent predictors of delirium incidence.

Peak 3.30pm cortisol (days 0-14)

Complete data were available for 82 participants and the model accounted for between 27% and 41% of the variance in delirium status. Table 5.14 shows the odds ratio and 95% confidence intervals for each of the predictor variables. This shows that only participant age was an independent predictor of delirium incidence, with NIHSS score at admission approaching significance (OR 1.18, p=0.06)

Ratio of peak morning: afternoon cortisol (days 0-14)

Complete data were available for 80 participants and the model accounted for between 34% and 52% of the variance in delirium status. Table 5.15 shows the odds ratio and 95% confidence intervals for each of the predictor variables. This shows that only age, NIHSS score on admission, IQCODE score and APACHE II score were independent predictors of delirium incidence.

Table 5.10 Results of the multivariate analysis with median 9 am cortisol as the predictor variable.

Variable	Odds ratio	95% CI	p
Age (years)	1.12	1.02-1.22	0.01
Sex (female)	1.66	0.47-5.86	0.43
NIHSS on admission	1.31	1.09-1.57	0.004
IQCODE score	1.71	0.66-4.41	0.27
Charlson Comorbidities Index	0.90	0.59-1.37	0.62
APACHE II	1.10	0.86-1.41	0.44
Median 9 am cortisol (day 0-7)	0.95	0.89-1.01	0.08

Table 5.11 Results of the multivariate analysis with median 3.30pm cortisol as the predictor variable

Variable	Odds ratio	95% CI	p
Age (years)	1.10	1.01-1.19	0.03
Sex (female)	1.61	0.45-5.74	0.46
NIHSS on admission	1.19	1.01-1.41	0.04
IQCODE score	1.88	0.71-4.94	0.21
Charlson Comorbidities Index	0.98	0.64-1.50	0.92
APACHE II	1.02	0.83-1.25	0.88
Median 3.30pm cortisol (day 0-7)	1.01	0.95-1.07	0.83

Table 5.12 Results of the multivariate analysis with the ratio of median 9am to 3.30pm cortisol levels as the predictor variable

Variable	Odds ratio	95% CI	p
Age (years)	1.08	0.99-1.18	0.06
Sex (female)	0.49	0.13-1.90	0.30
NIHSS on admission	1.20	1.02-1.41	0.03
IQCODE score	1.78	0.66-4.76	0.26
Charlson Comorbidities Index	0.96	0.63-1.48	0.86
APACHE II	1.08	0.87-1.34	0.50
Median morning: afternoon cortisol ratio (day 0-7)	0.46	0.18-1.18	0.11

Table 5.13 Results of the multivariate analysis with peak 9am cortisol (day 0-14) as the predictor variable

Variable	Odds ratio	95% CI	p
Age (years)	1.10	1.01-1.21	0.04
Sex (female)	0.44	0.11-1.71	0.24
NIHSS on admission	1.32	1.1-1.59	0.003
IQCODE score	3.44	1.03-11.5	0.05
Charlson Comorbidities Index	0.97	0.61-1.56	0.91
APACHE II	1.07	0.81-1.42	0.62
Peak 9am cortisol (day 0-14)	0.97	0.93-1.01	0.19

Table 5.14 Results of the multivariate analysis with peak 3.30 pm cortisol (day 0-14) as the predictor variable

Variable	Odds ratio	95% CI	p
Age (years)	1.12	1.02-1.23	0.02
Sex (female)	1.27	0.34-4.81	0.72
NIHSS on admission	1.18	0.99-1.41	0.06
IQCODE score	1.96	0.71-5.41	0.20
Charlson Comorbidities Index	0.97	0.63-1.50	0.88
APACHE II	1.01	0.81-1.25	0.97
Peak 3.30pm cortisol (day 0-14)	1.01	0.95-1.07	0.79

Table 5.15 Results of the multivariate analysis with the ratio of peak morning to afternoon cortisol (day 0-14) as the predictor variable

Variable	Odds ratio	95% CI	p
Age (years)	1.12	1.01-1.24	0.03
Sex (female)	1.69	0.36-7.80	0.50
NIHSS on admission	1.25	1.05-1.51	0.02
IQCODE score	3.98	1.11-14.20	0.03
Charlson Comorbidities Index	1.03	0.63-1.70	0.90
APACHE II	0.99	0.79-1.26	0.95
Peak morning : afternoon cortisol ratio (day 0-14)	0.77	0.36-1.65	0.50

5.3.10 Association between delirium and salivary cortisol over time

A generalised linear random effects model was fitted, using delirium (yes or no) as the outcome variable and cortisol levels over time as the predictor variables. Two participants (participants 2 and 71) were excluded from the analysis as they were extreme outliers on at least one of the assessment time points (see appendix 8). The total number of paired cortisol measurements and delirium assessments were 335 in the no delirium group and 130 in the delirium group. The model showed that the probability of developing delirium increased per unit (nmol/L) increase in both morning and afternoon cortisol, but neither of these effects reached statistical significance (p=0.70 and p=0.46 respectively). The probability of developing delirium decreased per unit increase in morning to afternoon cortisol ratio, and also decreased per week after stroke onset, but again neither of these effects reached statistical significance (p=0.79 and p=0.87 respectively).

Table 5.16 Results of the generalised linear random effects model, with delirium as the outcome variable, and cortisol levels over time as the predictor variables.

Variable	Odds ratio	95% CI	p
Morning cortisol (nmol/L)	1.02	0.84-1.19	0.70
Afternoon cortisol (nmol/L)	1.05	0.82-1.25	0.46
Ratio of morning to afternoon cortisol (nmol/L)	0.77	0.02-2.10	0.79
Time after stroke (weeks)	0.99	0.85-1.10	0.87

5.4 Discussion:

This study is the largest to date to examine the associations between cortisol levels and delirium after stroke. Afternoon cortisol levels were found to be higher in those diagnosed with delirium in the first 14 days after stroke, and afternoon cortisol levels also correlated with delirium severity. Morning cortisol levels were not associated with delirium diagnosis or severity in bivariate analyses. There was no evidence that cortisol was an independently associated with delirium, once confounders had been controlled for, although morning cortisol levels in the first 2 weeks after stroke approached statistical significance (OR=0.95, p=0.08). Only advancing age and NIHSS score were consistently independently associated with delirium in the multivariate analysis. Finally, there was no evidence from generalised linear random effects modelling to support a role for cortisol in the pathogenesis of delirium after stroke in the 1 year following acute stroke in this study.

A relationship between salivary cortisol and delirium after stroke remains conceivable. It is known that physiological and psychological stressors may precipitate delirium (MacLullich et al., 2008) and furthermore, we have shown in chapter 2 that acute stroke is associated with elevated cortisol levels, particularly in the first 7 days. As previously discussed, at present there are just five published studies which have investigated the relationship between cortisol levels after stroke and delirium (Fassbender et al., 1994, Gustafson Y, 1993, Marklund et al., 2004, Olsson, 1990, Olsson et al., 1992), with four out of five (n=207) reporting higher cortisol levels in those with delirium and one reporting no difference (n=23). However, all have a small sample size (median n=23, IQR18-85.5) and none of them measured either cortisol nor screened for delirium with the frequency or regularity of this study. Whilst we have failed to show an independent relationship between cortisol and delirium, there does seem to be a tendency towards higher cortisol in those with delirium. It must be acknowledged that the power calculation used to determine the sample size for this study, was based on an incidence of delirium of 40% (as found in the study by Gustafson and colleagues) (Gustafson Y, 1993). More recent studies have tended to find a lower incidence of delirium after stroke (around 25% in a recent systematic review (Shi et al., 2012)), which may partly be accounted

for by the advent of new treatments for acute stroke (such as thrombolysis) and the universal provision of stroke unit care. This means that this study had less power to detect the effect of cortisol on delirium, than was originally predicted. A larger study with greater power would be needed to establish whether cortisol truly has an independent effect on delirium status, or whether it is simply a marker of other factors, such as stroke severity. It must be noted that large studies in this patient population are difficult to do, because participants are often very unwell following stroke and yet must be assessed for delirium and have cortisol levels measured regularly. Proxy consent is often required (either because of the severity of stroke or because of pre-existing dementia) and this, along with the fact that a long-term follow-up period is desirable (because delirium can persist) make recruitment challenging. These difficulties are reflected in the small sample sizes in previous studies. Meta-analysis would also be challenging with the studies currently available, as methodologies employed vary considerably from study to study, particularly in the method of cortisol measurement.

Stroke severity (as measured by the NIHSS score) and age were both independently associated with delirium in this study. This is interesting, and supports the existing evidence with respect to predictors of delirium after stroke. The largest study of delirium after stroke to date (n=527) (Oldenbeuving et al., 2011) found that stroke severity and age were independent predictors of delirium (although age only became an independent predictor if brain atrophy was left out of the model, presumably because the two may co-vary), and as previously described these factors both have plausible pathophysiological mechanisms which might explain their consistent association with delirium. Age is associated with neurone loss, reduced blood flow to the brain, and reduced vascular density in the brain, all of which may increase susceptibility to delirium. Pathological processes in the brain such as those seen in vascular dementia or Alzheimer's dementia are also more common in the ageing brain, and these also increase the risk of delirium (Maldonado, 2013). Stroke severity is a complex variable, as the severity score may not always correlate with the actual size of the brain lesion, although in many cases it will. Furthermore, stroke severity is not necessarily a marker of overall illness severity, although again there is often a correlation between the two (and those with a large TACS are also more at risk of intercurrent illness, such as pneumonia). However, overall the stroke severity score is

a marker of the direct brain insult and subsequent brain tissue damage and loss (Mitsias et al., 2002), all of which are likely to increase the risk of delirium.

Only one previous study, by Ahmed and colleagues (Ahmed et al., 2004) examined salivary cortisol levels after stroke (rather than plasma or urinary levels). It found a mean salivary cortisol level (at admission) of 18.4nmol/L in the morning and 6.7nmol/L in the afternoon. In this study I found a mean morning level of 25.9nmol/L and a mean afternoon level of 17.7 nmol/L during the first week after stroke. The reason for the slightly higher levels in this study are not clear. It may partly be accounted for by the fact that collection devices and assays used differed, however assays in this study were calibrated to industry standards. Stroke severity is known to influence cortisol levels in a bidirectional fashion, in that those with very severe strokes in ICU have been shown to have low cortisol levels and lose the diurnal circadian rhythm , whereas more generally the more severe the stroke, the higher the cortisol level is (Barugh et al., 2014). The mean NIHSS score in the Ahmed study was 7, and the median NIHSS in this study was 5, indicating similar stroke severity. The Ahmed study was smaller (n=58) and the study participants were younger (mean age of 66, compared with 77 in this study), and although delirium wasn't reported, it's incidence is likely to have been lower, because participants were younger (and therefore less likely to have underlying risk factors for delirium such as pre-existing dementia).

There are some limitations to this study. Participants were recruited as soon after stroke as possible, however because the time of stroke onset was taken to be when the participant was last seen well, there were delays between this time and first assessment. There were also delays when proxy consent was required, as proxies, understandably, often required 24 hours or more to consider the study before granting consent. These factors meant that some participants were not assessed, nor did they have salivary cortisol measured until day 5 after stroke onset. Participants were not assessed every day following recruitment, rather they were assessed on alternate days. This methodology was used to reduce the burden of assessments for participants, however the chart-CAM and clinician and informant information was used to provide information about the days when formal assessments weren't scheduled. It is possible

that brief episodes of delirium were missed, although this could also be the case if participants were assessed daily. There was selection bias in the study, as proxies were more likely to decline consent compared with potential participants themselves, resulting in a bias towards selection of those with capacity at recruitment. The multivariate analysis presented in this chapter must be interpreted with caution. It is recommended that, as a guide, 10 cases of data for each predictor in the model are required, although this is something of an oversimplification, as the sample size required will ultimately depend on the size of the effect that is being investigated (Field 2009). As the effect size of cortisol on delirium after stroke was unknown, the simple rule of thumb of 10 cases for each predictor was adhered to. One method of correcting for multiple comparisons is to use a Bonferroni correction, whereby the p value which is taken to imply a significant relationship is taken to be 0.05 divided by the number of comparisons made (Field 2009). For the multivariate models presented in this chapter, applying a Bonferroni correction does not alter the overall result, but it does mean that none of the variables in the models would be significantly associated with the diagnosis of delirium. Finally, as discussed above, the sample size was small, although this remains the largest study to date of cortisol and delirium after stroke.

In summary, I have not found evidence of an independent association between salivary cortisol levels after stroke and the development of delirium. A tendency towards higher morning and afternoon cortisol levels in the 14 days after stroke in those who developed delirium was demonstrated, but the study did not have sufficient power to determine categorically whether these relationships are real. However, these results provide useful data which could be used to inform larger, definitive studies.

Chapter 6: Delirium, cognitive function (over 1 year) and functional outcome

6.1 Introduction

Delirium is a strong risk factor for the development of dementia in the elderly population in general (Davis et al., 2012), and in hospitalised general medical patients delirium has been shown to be an independent risk factor for the subsequent development of dementia (Rockwood et al., 1999). Dementia is common after stroke, affecting around 12% of those with a first ever stroke (when those with pre-existing dementia are excluded), and around 41% of stroke survivors overall (when those with recurrent stroke and those with pre-existing dementia are included) (Pendlebury, 2012a). When compared with controls, stroke survivors have around a nine-fold excess risk of dementia in the first year after their stroke (Pendlebury, 2012a). A recent large prospective cohort study of the general population of the United States of America (aged 45 or over, 23 572 participants, of whom 515 had an incident stroke during the 6 year study period) found that incident stroke was associated with an accelerated and persistent decline in global cognition and executive function and an acute decline in verbal memory and new learning ability (Levine et al., 2015). Delirium has been shown to be an independent predictor of cognitive decline 2 years after stroke in a study of 120 stroke patients, with those who developed delirium after stroke having between a five and seven fold increased risk of having dementia at follow-up (van Rijsbergen et al., 2011). However, it is important to note that participants in this study were only screened for delirium twice during the first week after stroke (and were then only monitored for presence of delirium if delirium was detected in the first week), meaning that delirium which developed later would not have been identified. Furthermore, only 50 participants were able to complete the 2 year follow-up due to high mortality rates in both groups, although the mortality rate was higher in the delirium group, with 65% dying before follow-up was completed. Other risk factors associated with poststroke dementia include advancing age, prior cognitive decline, brain atrophy and white matter changes on imaging and stroke severity (Pendlebury, 2012a).

Pre-existing dementia is a risk factor for development of delirium in the general population and also in stroke survivors (Fong et al., 2012, Pendlebury, 2012b). However the way in which delirium and dementia are linked is not clear. It may be that an episode of delirium unmasks previously unrecognised dementia, or that delirium itself causes permanent neuronal damage leading to dementia, or indeed that delirium is a marker of a predisposition or vulnerability to dementia (Fong et al., 2012). Longitudinal studies have shown that those with pre-existing Alzheimer's dementia who go on to develop delirium have a worse prognosis than those with dementia alone, in terms of mortality and cognitive decline (Fong et al., 2012). It is not clear if this is also true of stroke survivors, with pre-existing dementia (of Alzheimer's or vascular type).

Previous studies of delirium after stroke have found that those who developed delirium had higher rates of institutionalisation, and poorer functional outcome, when compared to those who did not develop delirium (McManus, 2009). This may be related to cognitive decline, but also may be due to physical frailty, both of which may preclude independent function and living.

This study examines the relationship between delirium after stroke (at any point during the 12 month study period) and cognitive function over the 12 months after stroke. It also investigates the functional outcome for participants at 1 month, 4 months and 12 months after stroke.

The hypothesis addressed in this chapter is: Delirium after stroke is associated with decrements in cognition in the 1 year after stroke.

6.2 Methods

95 participants with an acute stroke were recruited as described in chapter 4.

6.2.1 Baseline Cognitive Assessment

At recruitment (day 0-5 post stroke onset) the National Adult Reading Test (NART) was administered, to estimate crystallised intelligence (Nelson, 1991), the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) was completed by an informant and was used as a measure of premorbid cognitive impairment. Those with an average IQCODE score of <3.19 were deemed to have normal cognition (prior to stroke onset), those with a score of ≥3.19-3.43 were deemed to have evidence of pre-existing mild cognitive impairment and those with a score of ≥3.44 were deemed to have evidence of pre-existing dementia (Harwood et al., 1997, Li et al., 2012). The Montreal Cognitive Assessment (MoCA) was used as a brief test of global cognition (Nasreddine, 2005). Digit Span Forwards and Backwards was used to test attention and processing.

6.2.2 Follow-up Cognitive Assessments

Follow-up assessments were undertaken at 1, 4 and 12 months post stroke. Prior to each visit the participant's GP was contacted to ensure that the participant had not died, and that they were well enough to be visited. The participant was then contacted by telephone to arrange the visit, and a letter was sent in the post to confirm the time and date of this appointment. The participants then completed a cognitive test battery, which is outlined in detail in chapter 4, section 4.6. The battery took around 50 minutes to complete, but testing was stopped if participants became too fatigued or declined to continue. The Montreal Cognitive Assessment was also completed at follow-up. All follow-up visits took place in the participants own home (or place of residence if relevant, such as a nursing home). Most of the follow-up visits were conducted by me, however 54 out of 249 were conducted by one of three Community Research Nurses (Avril Cairns, Catherine Beveridge or Frances Docherty), during a six month period of the study when I was on maternity leave. The nurses were all trained in how to administer the cognitive tests by me, and all attended at least two home visits with me to observe, prior to performing one visit each themselves whilst I observed.

6.2.3 Follow-up delirium assessments

Participants were assessed for evidence of either persistent or new onset delirium at each follow-up visit (1 month, 4 months and 12 months), using the same methodology as was used during the initial post-stroke assessments (described in detail in chapter 4 section 4.4). Briefly, this involved assessment for the features of delirium using the CAM-ICU, DRS-R98, OSLA and DEL-box, the results of which were used to inform diagnosis based on DSM-IV criteria. The participant's next of kin, where available, was questioned about any episodes of altered cognition in the period between follow-up, and any episodes suspicious of delirium were discussed with the participant's GP (if they had been consulted). Any hospital admissions were noted and also discussed with the participant's GP thereafter. Delirium was diagnosed if CAM-ICU or the chart CAM (in the case of a retrospective diagnosis) was positive and DSM-IV criteria were fulfilled.

6.2.4 Functional outcome

At each of the follow-up assessments (1 month, 4 months and 12 months), the Nottingham Extended Activities of Daily Living Scale (Lincoln and Gladman, 1992) was completed for each participant. This is described in more detail in section 4.7, but briefly comprises 22 self-rated items about everyday functional ability. The test is scored out of a maximum of 22 points with a higher score indicating better functional ability. For those participants who were unable to complete the questionnaire themselves, a proxy was asked to complete it on their behalf. Information about place of residence was also recorded at each follow-up visit and length of stay in hospital was obtained from the hospital computerised records system.

6.2.5 Statistical analysis

Data were assessed for normality of distribution using histogram plots.
In order to explore the pattern of global cognitive change across the year of follow-up, median MoCA scores at each time point, for the whole cohort, for those who developed delirium at any point during the study and for those who did not develop delirium, were plotted on a line graph. In order to explore the effect of delirium severity on the trajectory of global cognition over 12 months, those who developed delirium were divided into four groups, based on the DRS-R98 score at the time of

delirium diagnosis (those with a score of <18, 18-21, 22-25 and >25) and a line graph of the MoCA trajectory for each group was plotted.
In order to explore the relationship between prior cognitive status (prior to stroke-normal, mild cognitive impairment or dementia, based on IQCODE score) and delirium (incident, persistent, resolved or never) at each time point, a Pearson chi-square analysis was performed.

In order to investigate the differences between those who did and those who did not develop delirium in terms of baseline cognition (Crystallised intelligence measured using the NART and pre-morbid cognition assessed using the IQCODE) and global cognition (assessed using the MoCA) group comparisons were made using the Mann-Whitney U test.

In order to explore the differences in cognitive test scores between the group who did and the group who did not develop delirium, comparisons were made using ANOVA (for the parametric data) or Kruskal-Wallis test (for the non-parametric data) at each time point. To account for the effect of active delirium at the time of testing, any participant with an active delirium was excluded from these analysis, but were included at subsequent time points, if their delirium has resolved (no participant was excluded for the whole of the follow-up). All analysis, except where otherwise stated, were performed using SPSS version 14 and version 19.

In order to explore the effects of delirium on the trajectory of the cognitive test scores over the 12 month study period, mixed effects multiple regression modelling was carried out by Dr Daniel Davis using the Stata 13.1 statistics package. Longitudinal change in cognitive outcome was estimated using random-effects models, where individuals were allowed to have random intercepts (cognitive score at first measurement) and random slopes (rate of change in cognition). The time metric was "time in study" and only linear relationships were considered. Covariance matrices were unstructured. The following covariates were considered for the intercept and slope: age, sex, NIHSS score on admission, IQCODE score, Charlson Comorbidities Index score and APACHE II score. Model fit was assessed using maximum likelihood estimates. Assumptions were checked by constructing Q–Q plots of the standardised residuals.

6.3 Results

95 participants were included in the study, 26 (27%) of whom developed delirium at some point during the 12 months following acute stroke. Baseline characteristics are summarised in chapter 5 section 5.1. Cognitive assessments were performed on all participants at the time of recruitment (days 3-5 post stroke), and were available for 80 (84%) participants at day 28, 83 (87%) participants at 4 months and for 73 (77%) participants at 12 months.

Of the 26 who developed delirium during the study, cognitive assessments were available for 20 (77%) at day 28, 21 (81%) at 4 months and 15 (58%) at 12 months. Of the remaining 69 who did not develop delirium throughout the course of the study, cognitive assessments were available for 60 (87%) at day 28, 62 (90%) at 4 months and 58 (84%) at 12 months. Reasons for not completing cognitive assessments are summarised in table 6.1. There were significant differences between the groups in terms of reasons for not completing cognitive testing. Those in the delirium group were more likely to have died, however those without delirium were more likely to be too unwell to complete testing at 1 month.

An episode of incident delirium (meaning a new diagnosis of delirium) at the follow-up visits was uncommon, in fact occurring only once throughout the whole study duration. This may be because I was required to ensure participants were well enough to participate (by contacting them, their carer or their GP) prior to visiting them. Persistent delirium was present in 8 participants at the 1 month follow-up, 15 had resolved delirium and the remaining 64 had never had delirium up to that point. At the 4 month follow-up, 2 participants had persistent delirium, 21 had resolved delirium and the remaining 64 had never had delirium. Finally at the 12 month follow-up, 1 participant had incident delirium, no-one had persistent delirium, 20 had resolved delirium and 63 participants had never had delirium. Those with persistent delirium at 1 month were more likely to have underlying mild cognitive impairment or dementia (as diagnosed by IQCODE score), however there was no significant relationship between delirium status and prior cognition found at 4 months and 12 months (table 6.2).

Table 6.1 Reasons for failure to complete cognitive assessments at each time interval, for those who did and those who did not develop delirium

Time post stroke	Delirium	No Delirium	p◊
1 month Total able to complete testing = 80	Too unwell n=3 Died n=2 Declined n=1	Too unwell n=6 Declined n=3	<0.001
4 months Total able to complete testing = 83	Died n=4 Declined n=1	Too unwell n=2 Declined n=5	<0.001
12 months Total able to complete testing = 73	Too unwell n=2 Died n=8 Declined n=1	Died n=4 Declined n=7	<0.001

◊Pearson chi-square

Table 6.2 Prior cognitive status, assessed by IQCODE score, for those who did and those who did not develop delirium

Time post stroke	Delirium status	Stratification based on IQCODE (N=87)			p◊
		Normal	MCI	Dementia	
1 month	Incident	0	0	0	0.015
	Persistent	1	0	7	
	Resolved	6	3	6	
	Never	35	11	18	
4 months	Incident	0	0	0	0.096
	Persistent	0	0	2	
	Resolved	7	3	11	
	Never	35	11	18	
12 months	Incident	0	0	1	0.113
	Persistent	0	0	0	
	Resolved	7	3	13	
	Never	35	11	17	

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly. IQCODE ≤3.19 = normal, 3.2-3.43 = mild cognitive impairment, MCI, ≥3.44 = dementia

◊Pearson chi-square

6.3.1 Delirium, functional outcome and length of stay after stroke

The median Nottingham ADL score (as a measure of functional outcome) improved over the course of the 12 month follow-up period, as would be expected due to recovery from the stroke and also attrition of those with more severe strokes (table 6.3). This improvement was even more marked in the group who developed delirium, which may have been due to resolution of delirium over time (the majority of delirium occurred in the first two weeks after stroke onset). At each time point, those who never developed delirium had significantly better functional outcome compared to those who developed delirium at some point.

There were significant differences between groups with respect to place of residence prior to stroke, with those who went on to develop delirium being more likely to require care at home or be in a nursing home, compared to those who didn't develop delirium. After stroke, those who developed delirium were again more likely to require care at home or require nursing home care compared to those who didn't develop delirium. Length of stay was significantly longer for those who developed delirium, than for those who did not.

Table 6.3 A group comparison of functional outcome and length of hospital stay between those who did and those who did not develop delirium

	Whole cohort Median (IQR)	**Delirium Median (IQR)**	**No Delirium Median (IQR)**	**p**
Nottingham ADL score (out of 22)	1 month: 11 (4-16) 4 months: 14 (6-19) 12 months: 15 (5.5-19.5)	1 month: 3.5 (1-9.75) 4 months: 5 (1.75-14.25) 12 months: 18 (3.75-18)	1 month: 12 (6.75-17) 4 months: 16 (10-20) 12 months: 18.5 (12-21)	<0.001[a] <0.001[a] 0.04[a]
Place of residence pre-stroke	Own home: n=81 Own home with POC: n=10 Sheltered housing: n=3 Nursing Home: n=1	Own home n= 17 Own home with POC n=7 Sheltered housing n=1 Nursing Home n=1	Own home n= 64 Own home with POC n=3 Sheltered housing n=2 Nursing Home n=0	0.006[b]
Place of residence at 1 month	Own home: n= 45 Own home with POC: n= 23 Sheltered Housing: n= 2 Residential Home: n= 0 Nursing Home: n= 2 Remain in hospital n= 21	Own home: n= 4 Own home with POC: n= 10 Sheltered Housing: n= 0 Residential Home: n= 0 Nursing Home: n= 2 Remain in hospital n= 8	Own home: n= 41 Own home with POC: n= 13 Sheltered Housing: n= 2 Residential Home: n= 0 Nursing Home: n= 0 Remain in hospital n= 13	<0.001[b]
Place of residence at 4 months	Own home: n= 49 Own home with POC: n= 26 Sheltered Housing: n= 2 Residential Home: n= 0 Nursing Home: n= 4 Remain in hospital n= 11	Own home: n= 4 Own home with POC: n= 10 Sheltered Housing: n= 0 Residential Home: n= 0 Nursing Home: n= 4 Remain in hospital n= 5	Own home: n= 45 Own home with POC: n= 16 Sheltered Housing: n= 2 Residential Home: n= 0 Nursing Home: n= 0 Remain in hospital n= 6	<0.001[b]
Place of residence at 12 months	Own home: n=51 Own home with POC: n=27 Sheltered Housing: n=2 Residential Home: n=2 Nursing Home: n=6	Own home: n=4 Own home with POC: n=9 Sheltered Housing: n=0 Residential Home: n=2 Nursing Home: n=5	Own home: n=47 Own home with POC: n=18 Sheltered Housing: n=2 Residential Home n=0 Nursing Home: n= 1	<0.001[b]
Length of stay (days)	12 (6-30)	26.5 (11-52)	10 (5.5-23)	0.002[a]

[a]Mann-Whitney U test
[b]Pearson chi-square

6.3.2 Baseline cognition, global cognition and delirium

There was no difference in crystallised intelligence (measured by the NART) between those who did and did not develop delirium (table 6.4). Those who developed delirium had significantly fewer years in full time education and were more likely to have evidence of prior mild cognitive impairment or dementia (as assessed by the IQCODE) when compared with those who did not develop delirium. MoCA scores were significantly lower in the delirium group compared with the no delirium group throughout the study period. There was an overall trend for improvement in MoCA scores throughout the year. This may, at least in part, be explained by a learning effect (because of repeated administration of a similar test), and attrition of those with the most severe cognitive impairment, as well as resolution of delirium and resolution of intercurrent acute illness such as infection.

Table 6.4 Baseline assessments of crystallised intelligence (NART), Prior cognition (IQCODE) and serial measurement of global cognition (MoCA) over the 1 year after stroke

	Whole cohort	**Delirium**	**No delirium**	**p◊**
NART (at baseline)	35(29.25-41) N=84	34.5 (25.25-40.25) N=18	36 (29.75-41.25) N=66	0.356
Years of education	11 (11-13) N=95	11 (10-12) N=26	11(11-14) N=69	0.036
IQCODE	3.25(3-3.69) N=87 ≥3.44: N=31 3.19-3.43: N=14 ≤3.18: N=42	3.13 (3-3.5) N=24 ≥3.44: N=14 3.19-3.43: N=5 ≤3.18: N=5	3.13(3-3.5) N=63 ≥3.44: N=17 3.19-3.43: N=13 ≤3.18: N=33	0.005 0.013[a]
MoCA	Baseline: 22 (17-25) N=93 Day 28: 24 (19-27) N=80 4 Months:26 (21-28) N=83 12 Months:26 (20-28) N=73	Baseline: 13.5 (5-19) N=24 Day 28: 18 (8.25-22.75) N=20 4 Months: 20 (9.5-26.5) N=21 12 Months: 20 (15-26) N=15	Baseline:24 (19.5-27) N=69 Day 28: 25 (21-27) N=60 4 Months: 26 (23-29) N=62 12 Months: 26 (21.75-29) N=58	<0.001 <0.001 0.001 0.004

IQCODE: Informant Questionnaire of Cognitive Decline in the Elderly. IQCODE ≤3.19 = normal, 3.2-3.43 = mild cognitive impairment, MCI, ≥3.44 = dementia

◊Mann Whitney U test
a Pearson's chi-square

6.3.3 Cognition and delirium in the 1 year after stroke

The cognitive battery was administered in its entirety at 1 month, 4 months and 12 months follow-up visits. However, there is a large amount of missing data for the battery, partly because of attrition due to death, declining to continue the study or moving away, as detailed in table 6.1 previously. Furthermore, not all participants completed all of the tests at each visit. Reasons for failing to complete testing were fatigue, participants declining to complete a particular test, significant dementia (resulting in the participant being unable to understand the instructions for the test) and poor eyesight (deteriorated since recruitment). Attrition was greater in the delirium group, than in the group who never developed delirium. The results of each cognitive test (median and IQR) are presented as raw scores (unadjusted for NART and IQCODE). Table 6.5 shows the raw scores for each test at each time point, for the whole cohort, and also for those who did and did not develop delirium. Tables 6.6 to 6.8 show the cognitive tests for these three groups at individual time points (1 month, 4 months and 12 months). In order to examine the influence of an episode of delirium on future cognition, group differences were examined between participants who had delirium during the study period and those who did not. In order to account for the effect of an acute episode of delirium on cognitive testing, any participant with an active delirium at the time of follow up has been excluded from this analysis (this applies to 8 participants at 1 month, 2 at 4 months and 1 at 12 months).

6.3.3.1 Verbal fluency

Median verbal fluency scores for both letter (FAS) and animal naming were stable throughout the study period (1 month to 12 months) in the whole cohort. At each follow-up there was a significant difference between the group who had resolved delirium and those who never developed delirium, determined by post hoc analysis.

6.3.3.2 Visual reproduction

Taking the cohort as a whole, there was a trend towards improvement in all scores in the visual reproduction battery. This may be accounted for by a learning effect (as the test was identical at each follow-up assessment), but may also be accounted for by improvement in those who recovered from delirium and attrition (due to death) of those who were frailest and

had an underlying cognitive impairment. In the delirium group there was a small improvement in immediate and delayed recall over the 1 year, however there was a small deterioration in the percentage retention test. It must be noted that the numbers of participants completing this test in the delirium group are small (N of between 4 and 10). In the no delirium group, scores for all aspects of visual reproduction tended to improve over the course of the year, which may be accounted for by the reasons previously discussed.

6.3.3.3 HVLT-R List Learning

The scores for total recall for HVLT-R list learning improved in all groups (whole cohort, delirium and no delirium) over the 1 year follow-up period. This may in part have been a learning effect, as although different word lists were used at each follow-up time point, strategies which improve performance (such as grouping categories together) may have been learned. The discrimination index score section of the test stayed relatively stable throughout the year taking the cohort as a whole and in the group that never developed delirium. There was a tendency to improvement in the delirium group, which may reflect a learning effect and attrition of those with the most severe cognitive impairment. Post hoc analysis showed that there was a significant difference between the delirium and no delirium groups for both parts of the test at 1 month and 4 months, and also for the total recall test at 12 months (with the delirium group scoring significantly less well), however there was no significant difference between the groups for the discrimination index test at 12 months. Overall this would indicate poorer verbal memory in the delirium group compared with the no delirium group.

6.3.3.4 Victoria Stroop

The interference and difference scores remained stable for the whole cohort throughout the course of the 1 year follow-up period. Taking the group who developed delirium at some point during the study period separately, there was an overall decrease in the difference score at the 4 month follow-up visit, a pattern which was not seen in the group who never had delirium. The reason for this deterioration is not clear, and there was an improvement seen at the 12 month follow-up. However, it must be noted that the number of participants was only 13 in the delirium group at 4 months, and so a poor performance by a few participants would have a large effect on the group median score.

6.3.3.5 Digit span (forwards and backwards)

Scores for digit span forwards and backwards remained relatively stable in the whole cohort throughout the course of the year. At 1 month there was a significant difference in digit span backwards scores between those who developed delirium at some point and those who did not (with those in the delirium group having lower scores). There was no significant difference in digit span forwards scores. At 4 months and 12 months there was no significant difference between the two groups in either digit span forwards or digit span backwards scores.

6.3.3.6 Digit Symbol Substitution Test (DSST)

Scores on the DSST improved over time in the whole cohort, and taking the delirium and no delirium groups separately, each group showed an improvement over time. This may have been a learning effect, as the same test was administered at each follow-up, and may also be due to attrition of those with more severe cognitive impairment. Scores were significantly lower in the delirium group compared with the no delirium group at all 3 follow-up time points. This would indicate poorer attention and processing speed in the delirium group.

Table 6.5 Cognitive test scores (unadjusted), for the whole cohort, for those who developed delirium and those who did not, over time.

Cognitive test	**Whole cohort –median score (IQR)**				**Delirium-median score (IQR)**				**No delirium-median score (IQR)**			
	Day 3-5	1 month	4 months	12 months	Day 3-5	1 month	4 months	12 months	Day 3-5	1 month	4 months	12 months
MoCA (out of 30)	22 (17-25) N=93	24 (19-27) N=80	26 (21-28) N=83	26 (20-28) N=73	13.5 (5-19) N=24	18 (8.25-22.75) N=20	20 (9.5-26.5) N=21	20 (15-26) N=15	24 (19.5-27) N=69	25 (21-27) N=60	26 (23-29) N=62	26 (21.75-29) N=58
Verbal Fluency Letters (FAS)	-	7 (4-11) N=83	9 (6-12) N=80	9 (5-14) N=70	-	4 (1-6) N=19	4.5 (1.75-7.25) N=18	6 (4-9) N=15	-	8.5 (5-12) N=64	9.5 (7-13) N=62	10 (6-15) N=55
Verbal Fluency Animals	-	12 (7-15) N=83	12 (7-16) N=80	12 (8-17) N=71	-	6 (3-9) N=19	6 (2.75-10.5) N=18	8 (5-12) N=15	-	13.5 (10-16) N=64	14 (9-17) N=62	13.5 (9-19.75) N=56
Visual Reproduction-immediate recall	-	59.5 (39.5-73) N=54	59 (43-77) N=53	67 (45-79) N=60	-	40 (0-53) N=5	52 (20.75-67.25) N=10	52 (17.5-70.5) N=10	-	60 (42.5-74) N=49	62.5 (45-82.5) N=50	69.5 (51.25-79.75) N=49

Visual reproduction-delayed recall	-	18.5 (8-36.5) N=52	22 (8-51) N=55	34 (12.5-58.75) N=61	-	4 (0-23) N=4	5 (0-24.5) N=10	7.5 (0-38.5) N=10	-	20 (9-43) N=48	39 (9-54.25) N=50	35 (17-62) N=50
Visual Reproduction-% retention	-	35.5 (19.25-53.75) N=52	47.5 (15.75-68.5) N=54	53 (26-80.75) N=60	-	31.5 (0-69.25) N=6	8 (0-35.5) N=10	14 (0-47.25) N=10	-	38.5 (22.75-60) N=48	56 (24-74) N=50	55 (38-82) N=49
HVLT-R List Learning- Total Recall◊	-	13 (9-18) N=73	16 (11-19) N=70	18 (13-23) N=65	-	7 (3-12) N=15	10 (8-17) N=15	15 (8-20) N=12	-	15 (10-19) N=59	16 (12-19.5) N=61	18 (13-23) N=53
HVLT-R List Learning-Discrimination Index◊	-	9 (6-10) N=68	9 (7-11) N=70	9 (7-11) N=62	-	6 (2.5-9) N=13	7 (4.75-9) N=14	9 (7.75-9.25) N=10	-	9 (7-10) N=55	10 (8-11) N=56	9.5 (7-11) N=52
Victoria Stroop difference score	-	16 (9.5-27.3) N=69	17.5 (7.25-24) N=66	15 (6.9-21.14) N=62	-	20.5 (13.5-44.25) N=10	7 (-15-36.5) N=13	16 (-2-37) N=11	-	15.5 (9.75-26.4) N=58	17.5 (11.5-24) N=54	15 (9-20) N=51
Victoria Stroop interference score	-	1.56 (1.33-1.86) N=69	1.66 (1.3-2.0) N=66	1.57 (1.34-1.85) N=62	-	1.5 (1.28-2.17) N=10	1.15 (0.52-1.96) N=13	1.56 (0.96-1.8) N=11	-	1.6 (1.37-2.02) N=58	1.67 (1.34-2.02) N=54	1.57 (1.38-1.85) N=51
Digit Span Forwards	9 (8-11) N=93	10 (8-12) N=85	9 (8-12) N=81	9 (8-11) N=72	8 (1.75-10.75) N=24	9 (6.5-11) N=21	9 (8-11) N=20	8.5 (7-10) N=16	8 (1.75-10.75) N=69	10 (8-12) N=64	10 (8-12) N=61	9 (8-11) N=57

Digit Span Backwards	4 (3-6) N=93	5 (3-7) N=85	5 (4-6) N=81	6 (4-7) N=72	3 (0-5) N=24	3 (2-6) N=21	4 (3-6) N=20	5 (2-6.75) N=16	5 (3-6) N=69	5 (4-7) N=64	5 (4-6) N=61	6 (4-7) N=57
DSST	-	25 (17.5-33.5) N=49	27 (18-38.75) N=56	31 (20.25-41) N=52	-	12 (0-22) N=7	18.5 (6-23.25) N=6	21.5 (13.5-30.75) N=8	-	26.5 (19-35.75) N=42	27 (18-40) N=50	34.5 (21.25-41.75) N=44

◊Mean and SD, as normally distributed

Table 6.6 Group differences in raw cognitive test scores at 1 month between participants who had delirium at any point and those who never developed delirium, excluding those with active delirium at the 1 month assessment

Cognitive test	Whole cohort – median score (IQR)	Delirium-median score (IQR)	No delirium-median score (IQR)	p value
MoCA (out of 30)	24 (19-27) N=80	18 (8.25-22.75) N=20	25 (21-27) N=60	<0.001[b]
Verbal Fluency Letters (FAS)	7 (4-11) N=83	7 (4-11) N=83	8.5 (5-12) N=64	<0.001[b]
Verbal Fluency Animals	12 (7-15) N=83	6 (3-9) N=19	13.5 (10-16) N=64	<0.001[b]
Visual Reproduction-immediate recall	59.5 (39.5-73) N=54	40 (0-53) N=5	60 (42.5-74) N=49	0.042[b]
Visual reproduction-delayed recall	18.5 (8-36.5) N=52	4 (0-23) N=4	20 (9-43) N=48	0.079[b]
Visual Reproduction-% retention	35.5 (19.25-53.75) N=52	31.5 (0-69.25) N=6	38.5 (22.75-60) N=48	0.092[b]
HVLT-R List Learning- Total Recall◊	13 (9-18) N=73	7 (3-12) N=15	15 (10-19) N=59	<0.001[a]
HVLT-R List Learning-Discrimination Index◊	9 (6-10) N=68	6 (2.5-9) N=13	9 (7-10) N=55	0.027[a]
Victoria Stroop difference score	16 (9.5-27.3) N=69	20.5 (13.5-44.25) N=10	15.5 (9.75-26.4) N=58	0.204[b]
Victoria Stroop interference score	1.56 (1.33-1.86) N=69	1.5 (1.28-2.17) N=10	1.6 (1.37-2.02) N=58	0.973[b]
Digit Span Forwards	10 (8-12) N=85	9 (6.5-11) N=21	10 (8-12) N=64	0.05[b]
Digit Span Backwards	5 (3-7) N=85	3 (2-6) N=21	5 (4-7) N=64	0.034[b]
DSST	25 (17.5-33.5) N=49	12 (0-22) N=7	26.5 (19-35.75) N=42	0.003[b]

◊Mean and SD as normally distributed
[a]Kruskal-Wallis test (post-hoc, pairwise comparisons)
[b] ANOVA (post-hoc Dunnetts test)

Table 6.7 Group differences in raw cognitive test scores at 4 months between participants who had delirium at any point and those who never developed delirium, excluding those with active delirium at the 4 month assessment

Cognitive test	Whole cohort – median score (IQR)	Delirium-median score (IQR)	No delirium-median score (IQR)	p value
MoCA (out of 30)	26 (21-28) N=83	20 (9.5-26.5) N=21	26 (23-29) N=62	0.001[a]
Verbal Fluency Letters (FAS)	9 (6-12) N=80	4.5 (1.75-7.25) N=18	9.5 (7-13) N=62	<0.001[b]
Verbal Fluency Animals	12 (7-16) N=80	6 (2.75-10.5) N=18	14 (9-17) N=62	<0.001[b]
Visual Reproduction-immediate recall	59 (43-77) N=53	52 (20.75-67.25) N=10	62.5 (45-82.5) N=50	0.142[b]
Visual reproduction-delayed recall	22 (8-51) N=55	5 (0-24.5) N=10	39 (9-54.25) N=50	0.009 [b]
Visual Reproduction-% retention	47.5 (15.75-68.5) N=54	8 (0-35.5) N=10	56 (24-74) N=50	0.004 [b]
HVLT-R List Learning- Total Recall◊	16 (11-19) N=70	10 (8-17) N=15	16 (12-19.5) N=61	<0.001[b]
HVLT-R List Learning-Discrimination Index◊	9 (7-11) N=70	7 (4.75-9) N=14	10 (8-11) N=56	0.019[b]
Victoria Stroop difference score	17.5 (7.25-24) N=66	7 (-15-36.5) N=13	17.5 (11.5-24) N=54	0.365 [b]
Victoria Stroop interference score	1.66 (1.3-2.0) N=66	1.15 (0.52-1.96) N=13	1.67 (1.34-2.02) N=54	0.276 [b]
Digit Span Forwards	9 (8-12) N=81	9 (8-11) N=20	10 (8-12) N=61	0.109 [b]
Digit Span Backwards	5 (4-6) N=81	4 (3-6) N=20	5 (4-6) N=61	0.016 [b]
DSST	27 (18-38.75) N=56	18.5 (6-23.25) N=6	27 (18-40) N=50	0.034 [b]

◊Mean and SD as normally distributed
[a]Kruskal-Wallis test (post-hoc, pairwise comparisons)
[b] ANOVA (post-hoc Dunnetts test)

Table 6.8 Group differences in raw cognitive test scores at 12 months between participants who had delirium at any point and those who never developed delirium, excluding those with active delirium at the 12 month assessment

Cognitive test	Whole cohort – median score (IQR)	Delirium-median score (IQR)	No delirium-median score (IQR)	p value
MoCA (out of 30)	26 (20-28) N=73	20 (15-26) N=15	26 (21.75-29) N=58	0.004[b]
Verbal Fluency Letters (FAS)	9 (5-14) N=70	6 (4-9) N=15	10 (6-15) N=55	0.005 [b]
Verbal Fluency Animals	12 (8-17) N=71	8 (5-12) N=15	13.5 (9-19.75) N=56	0.001[b]
Visual Reproduction-immediate recall	67 (45-79) N=60	52 (17.5-70.5) N=10	69.5 (51.25-79.75) N=49	0.09 [b]
Visual reproduction-delayed recall	34 (12.5-58.75) N=61	7.5 (0-38.5) N=10	35 (17-62) N=50	0.013 [b]
Visual Reproduction-% retention	53 (26-80.75) N=60	14 (0-47.25) N=10	55 (38-82) N=49	0.006 [b]
HVLT-R List Learning-Total Recall◊	18 (13-23) N=65	15 (8-20) N=12	18 (13-23) N=53	0.029[a]
HVLT-R List Learning-Discrimination Index◊	9 (7-11) N=62	9 (7.75-9.25) N=10	9.5 (7-11) N=52	0.349 [a]
Victoria Stroop difference score	15 (6.9-21.14) N=62	16 (-2-37) N=11	15 (9-20) N=51	0.949 [b]
Victoria Stroop interference score	1.57 (1.34-1.85) N=62	1.56 (0.96-1.8) N=11	1.57 (1.38-1.85) N=51	0.227 [b]
Digit Span Forwards	9 (8-11) N=72	8.5 (7-10) N=16	9 (8-11) N=57	0.252 [b]
Digit Span Backwards	6 (4-7) N=72	5 (2-6.75) N=16	6 (4-7) N=57	0.097 [b]
DSST	31 (20.25-41) N=52	21.5 (13.5-30.75) N=8	34.5 (21.25-41.75) N=44	0.021 [b]

◊Mean and SD as normally distributed
[a]Kruskal-Wallis test (post-hoc, pairwise comparisons)
[b] ANOVA (post-hoc Dunnetts test)

6.3.4 Trajectory of global cognition over 12 months, controlled for the effect of delirium

As can be seen in figure 6.1 there was a trend for improvement in MoCA score for the whole cohort over the 12 month follow-up period. When stratified into groups based on delirium severity (figure 6.2) all except those in the worst severity group showed an improvement in global cognition. Those with the worst severity scores showed a decline, however there was significant attrition from this group, such than n=2 at 12 months. Those with a higher DRS-R98 score (indicating more severe delirium) had a significantly lower MoCA score when compared to those with lower DRS-R98 scores at baseline, 1 month and 4 months, but this effect was not significant at 12 months (table 6.9).

In a mixed effects multiple regression model, the rate of change per day for the whole cohort over time was 0.003 points per day (which is 0.09 points per month, and 1.09 points for the year) increase in MoCA (95% CI 0.0005-0.006, p=0.02) score. Table 6.10 shows that each episode of delirium was associated with a 5 point decrease in the MoCA score overall (95% CI -7.07- -3.78, p<0.001), but taking the whole group who had delirium, there was no significant effect on the rate of change per day of the MoCA (Coefficient 0.001, 95% CI -0.002-0.004, p=0.48). This means that delirium doesn't have a significant effect on the rate of change of the MoCA score, but does have a significant effect on the MoCA score overall.

Table 6.9 Group comparisons of MoCA scores, as a measure of global cognition, at baseline, 1 month, 4 months and 12 months, stratified by DRS-R98 score.

Time point	MoCA score for those with DRS-R98 <18 Median (IQR)	MoCA score for those with DRS-R98 18-21 Mean*	MoCA score for those with DRS-R98 22-25 Median (IQR)	MoCA score for those with DRS-R98 >25, Mean (SD)*	p◊
Baseline	18 (15.5-23.5) N=8	14 N=1	12 (7-19.3) N=10	2.6 (4.8) N=5	0.009
1 month	24 (19.3-25.8) N=8	20 N=1	13 (4.3-19.3) N=8	4.7 (8) N=3	0.023
4 months	26.5 (20.8-27.8) N=8	18 N=2	19.5 (8-25.3) N=8	6 (5.5) N=2	0.012
12 months	25.5 (17.5-26.5) N=7	19 N=2	21.5 (17-27) N=4	4.5 (5) N=2	0.083

*Mean used, rather than median, when n=2, raw data provided only when n=1.
◊Kruskal-Wallis test

Table 6.10 Mixed effects multiple regression models showing the effect of delirium on MoCA scores and trajectory of MoCA scores in the 12 months after stroke.

Variable	Coefficient	95% CI	p
Delirium (effect on overall MoCA score)	-5.42	-7.07- -3.78	<0.001
Delirium (effect on MoCA trajectory)	-0.02	-0.06-0.02	0.37

Figure 6.1 A line graph showing the trajectory of global cognition, measured using the Montreal Cognitive Assessment, over 12 months for the whole cohort and for those who did and those who did not develop delirium.

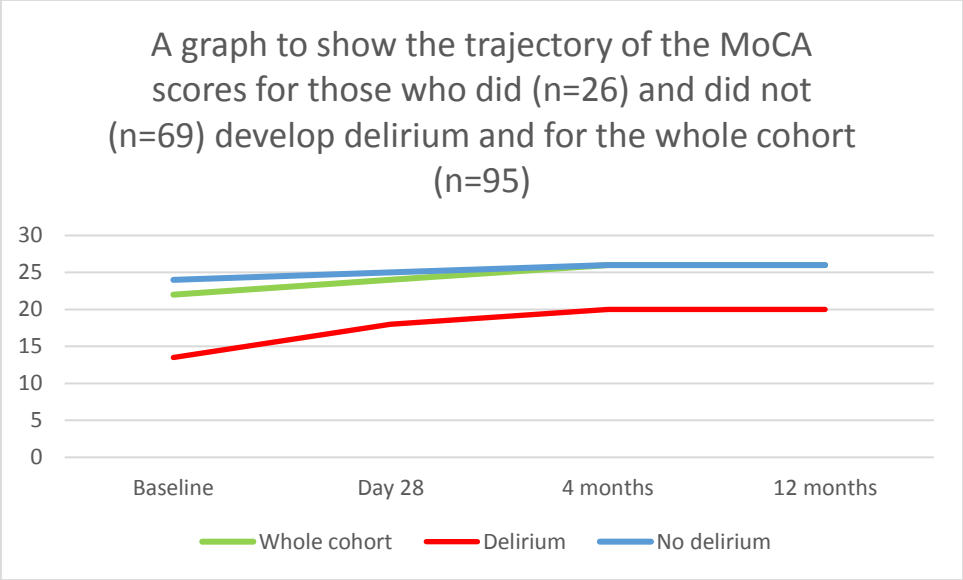


Figure 6.2 A line graph showing the trajectory of global cognition, stratified by delirium severity (DRS-R98), over 12 months

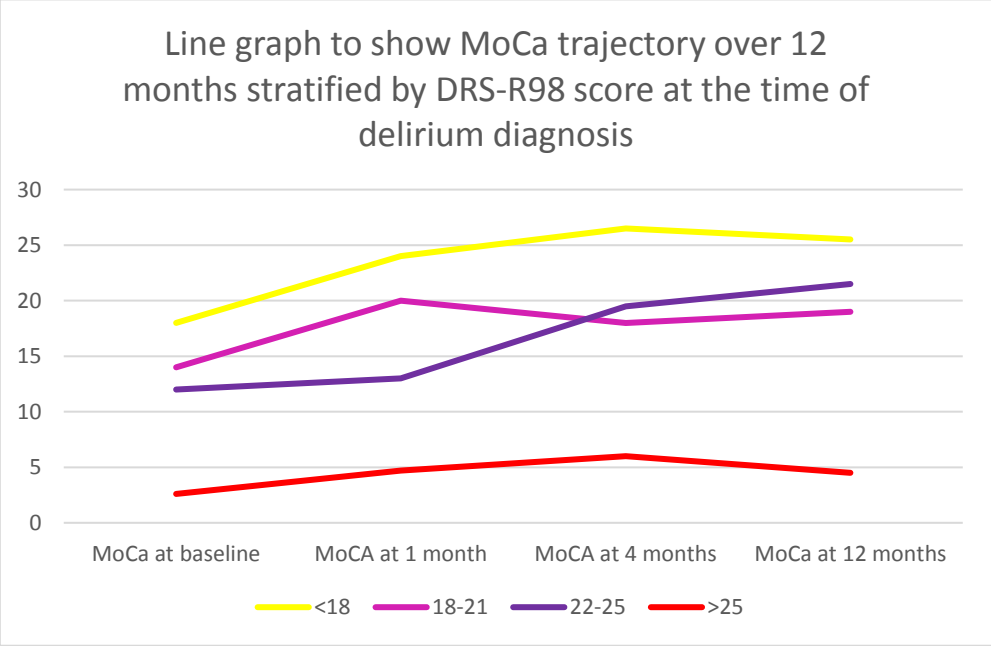
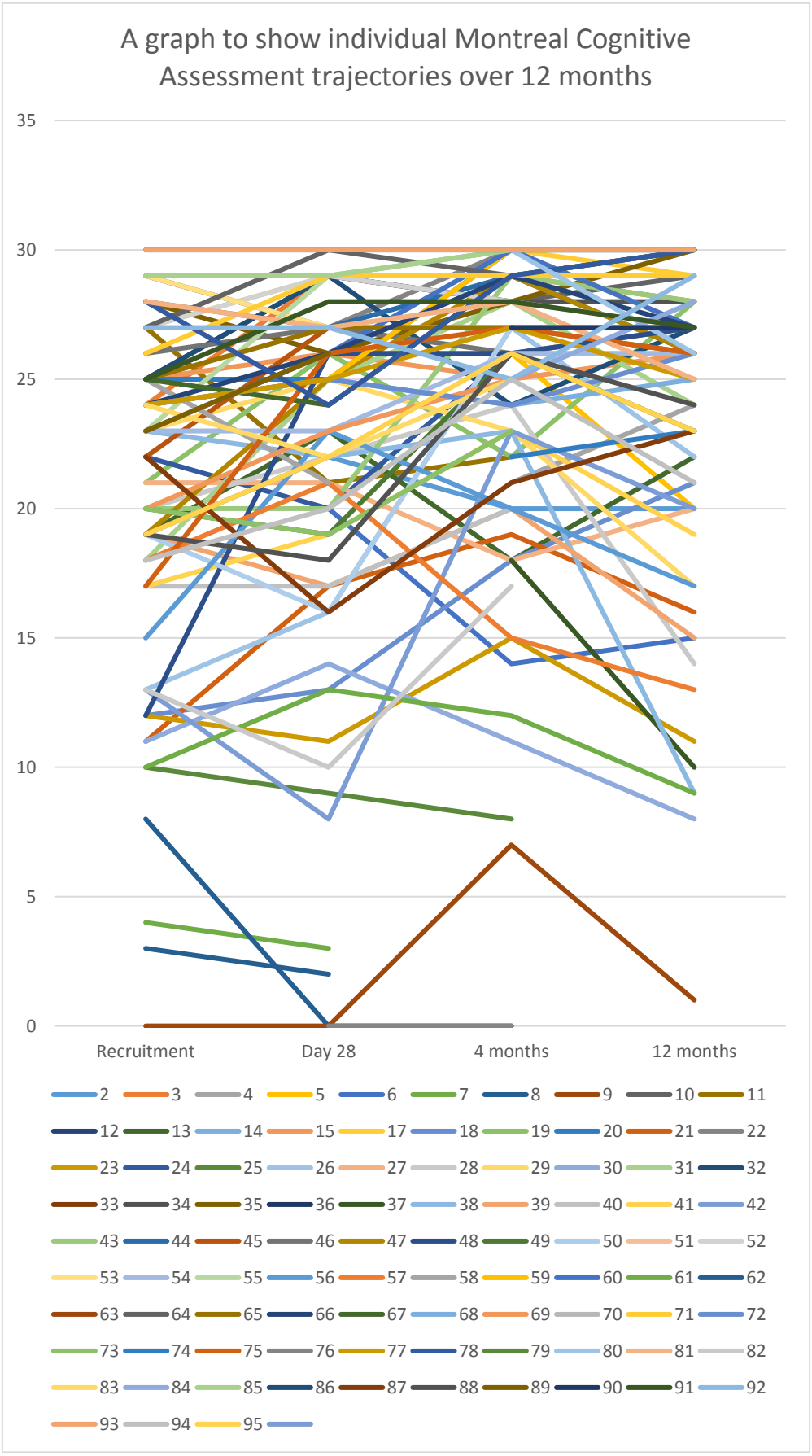


Figure 6.3 A graph to show all 95 individual Montreal Cognitive Assessment trajectories over 12 months



6.3.5 Trajectory of neuropsychological test battery scores over 12 months, controlled for the effect of delirium.

As can be seen in table 6.11, in mixed effects multiple regression models, the presence of delirium did not have a statistically significant effect on the trajectory of any of the neuropsychological test scores over time, although the HVLT-R list learning test approached statistical significance (p=0.07).

Table 6.11. Mixed effects multiple regression models showing the effect of delirium on the trajectory of each neuropsychological test score in the 12 months after stroke.

Variable	Coefficient	95% CI	p
Digit span	-0.056	-0.263-0.151	0.60
Verbal fluency	0.342	0.135-0.550	0.46
HVLT-R List Learning	-0.150	-0.31-0.009	0.07
Visual reproduction	-1.040	-3.21-1.13	0.35
Victoria Stroop	-0.022	-0.60-0.015	0.24
DSST	-0.126	-0.785-0.533	0.71

6.4 Discussion

This study found that delirium after stroke was associated with a higher IQCODE score (indicating presence of a pre-existing cognitive impairment or dementia), and fewer years in formal education. There was no difference in crystallised intelligence, measured by the NART (as a marker of pre-morbid IQ) between those who did and those who did not develop delirium. Global cognition, measured by the MoCA, was significantly poorer in the delirium group at each time point throughout the 12 months after stroke. However, there was a trend towards improvement in MoCA scores in the whole cohort throughout the 12 month follow-up, with the exception of those who developed the most severe delirium (rated by the DRS-R98 score). Those with a more severe delirium had significantly lower MoCA scores at baseline, 1 month and 4 months compared to those with lower severity scores. The presence of delirium at any point during the 12 month follow-up did not affect the rate of change of the MoCA scores over the 12 months after stroke. Finally, being diagnosed with delirium at any point during the study did not affect the rate of change of the neuropsychological test battery scores over the 12 months after stroke. As would be expected based on previous studies, those who developed delirium had a worse functional outcome, longer length of hospital stay and were more likely to require institutional care or a package of care at home, compared with those who did not develop delirium.

In this cohort, delirium was common, occurring in 27% of participants. Although the majority of delirium occurred in the first 7 days post stroke, persistent delirium was seen in 7 participants at the 1 month follow up and in 2 participants at the 4 month follow up. This illustrates the importance of careful assessment for delirium alongside more general cognitive testing at follow up in longitudinal studies of cognition after stroke, as delirium may significantly confound results if not recognised. Previous longitudinal studies of cognition after stroke have tended not to assess for delirium at any follow-up assessments (van Rijsbergen et al., 2011, Pendlebury, 2012a).

There was a trend for cognitive function to improve over the course of the 12 months after stroke. There are a number of possibilities as to why this may have been the case. Firstly, attrition was greatest in the delirium group and was highest of all in the subset of participants who had the highest delirium severity scores (the only group in whom global cognition

deteriorated over the 12 months). Attrition of these participants will skew the results. However, as can be seen in figure 6.3, many participants did complete the 12 month follow-up period and did show improvements in global cognitive function. There may have been a learning effect for the cognitive tests over time, which could account for some of the improvement seen. Care was taken to use different versions of tests, where available, however participants may have learned strategies for cognitive testing which can be applied to any version of the test. There may also have been an effect of recovery from acute illness, be that delirium, intercurrent infection and the stroke itself, all of which may have contributed to the improvement seen.

Previous studies of the trajectory of cognitive changes after stroke have tended to find an increase in dementia burden (with an associated decrease in cognitive function test scores) in the cohorts over time (Pendlebury, 2012a), although few studies have reported ascertainment of dementia rates after the first year post stroke. As previously discussed, a significant weakness of the majority of these studies is the failure to assess participants for delirium, which as we have shown, is a confounder. Just 3 previous studies have investigated cognitive changes after stroke in relation to delirium; Henon and colleagues (Henon et al., 1999) recruited 202 participants with stroke, assessed them for delirium during their in-patient stay and then performed MMSE as a measure of global cognition at 6 months. They did not ascertain evidence of delirium in the time between discharge and 6 months. Sheng and colleagues (Sheng et al., 2006) recruited 156 participants, who were assessed for delirium at baseline, and were followed-up for cognitive assessment, but again not for delirium assessment, at 1 month, 6 months and 12 months. Finally van Rijsbergen and colleagues (van Rijsbergen et al., 2011) followed up 50 stroke patients 2 years after stroke onset and performed neuropsychological testing, but again there was no ascertainment of episodes of delirium in the intervening time. All three studies found that delirium after stroke was associated with adverse outcomes. Henon and colleagues found an association between delirium and poorer functional outcome, van Rijsbergen found delirium to be an independent predictor of severe cognitive impairment at 2 years and Sheng and colleagues found delirium to be associated with higher mortality and poorer functional outcome. As briefly discussed in the introduction, a large population based study (Levine et al., 2015) of cognitive changes after stroke (n=515), recently found an acute decline in cognition following incident stroke, and also a persistent cognitive decline over 6 years post-stroke. However, again no assessments for delirium were performed post-stroke, and those with cognitive impairment

prior to stroke were excluded from the analysis. This is important as I have found that those with pre-existing cognitive impairment were more likely to develop delirium after stroke, but also showed improvement in global cognition, at the same rate of change as those who never developed delirium.

Overall, these findings, if reproduced by future larger studies, have important implication for stroke survivors and for clinicians, as cognitive impairment is one of the reasons that patients are discharged to institutional care (Hajek et al., 2015), rather than to their own home. The potential for improvement in global cognition between the time of first assessment after stroke and 12 month follow-up, found in this study, as well as the persistent nature of delirium in some cases, means that caution should be used in making such decisions, and ideally, these decisions should be revisited at least 4 months after stroke onset, when there may have been improvement in cognition, and when persistent delirium is likely to have resolved.

There are some limitations to this study which should be acknowledged. As has been discussed elsewhere in this thesis, the time from stroke onset until first assessment for presence of delirium was up to 5 days post-stroke (median day 3, IQR 3-5). This was because of delays in the recruitment process for those requiring proxy consent, but was also due to delays in presentation to hospital for some participants. This may have resulted in an underestimate of prevalent and also incident delirium, however every effort was made to ascertain if features of delirium had been present in the time prior to recruitment. There was more attrition in the delirium group, compared with the non-delirium group. From the literature, it is clear that this is inevitable, as those who develop delirium after stroke have higher mortality rates (McManus, 2011), however this does mean that there will be some bias towards those who are more robust, for the 1 year cognitive data. Furthermore, when those with delirium were stratified into levels of severity, based on the DRS score at the time of delirium onset, it became clear that those with more severe delirium had worse outcomes not only in terms of cognition but also had higher mortality rates. This meant that for those with severe delirium, the numbers remaining at 12 months were very small. It must also be acknowledged that although the study found that being diagnosed with delirium at any point in the study did not affect the trajectory of the neuropsychological test battery scores, these

tests were only performed at the follow-up assessments, at which point the majority of those with delirium had recovered. The study size is relatively small, taken in the context of stroke studies in general, however only 3 previous studies have investigated cognition after stroke longitudinally and investigated the effects of delirium on the long-term cognitive outcome, as previously discussed, all of which had methodological weaknesses. As discussed in section 5.4, the multivariate analysis should be interpreted with caution. The number of variables entered into the model did not exceed 1 per 10 cases of data, however the effect size of delirium on the cognitive trajectory was not considered when building the models. As discussed in section 5.4, a Bonferroni correction may be used to account for multiple comparisons, but once again when this was applied to the data presented in this chapter, the main findings were not altered. There is missing data in the study, because of attrition, and also because participants were not all able to complete every test at every visit. Finally the IQCODE was used as a screening tool for pre-morbid cognitive decline, as it was not possible to perform pre-morbid cognitive testing because of the nature of stroke. This does mean that the study is not able to determine the cognitive trajectory for participants from before stroke onset up to the 12 month follow-up, and so although improvement was seen between the first cognitive assessment (post-stroke) and 12 month follow-up, there may have been a deterioration in cognition in the cohort overall if we were able to compare the 12 month results with cognitive function pre-stroke.

In summary, this study has shown that delirium after stroke is common and may persist for up to 4 months after stroke onset. Global cognitive function tended to improve over the course of the 1 year after stroke, and the improvement in cognition showed a trend towards being more marked in the group who developed delirium at some point in the study, compared with those who never developed delirium, although this effect was not statistically significant. This overall trend towards improvement in cognitive function may be due to a learning effect for the cognitive tests, attrition of the frailest, most cognitively impaired participants and resolution of delirium during the course of the follow-up period.

Chapter 7: Cortisol levels, delirium and cognitive function in the 1 year after stroke

7.1 Introduction

There is broad agreement amongst the majority of studies of cortisol and its relationship to cognition (in non-stroke patients) that there is an association between elevated cortisol levels (measured in blood, saliva or cerebrospinal fluid) and poorer cognitive function, although there is considerable heterogeneity in study methodology.

Three population-based studies have investigated the associations between cortisol and cognition: the Baltimore Memory Study (Lee et al., 2007), the KORA-Age Study (Johar et al., 2015) and the Rotterdam Study (Schrijvers et al., 2011), however none of these specifically included stroke participants. The Baltimore Memory Study measured salivary cortisol in 967 community dwelling adults, aged 50-70. Four samples were taken during one visit, one before cognitive testing, one during testing, one after testing and one at the conclusion of the visit. In multivariate analysis, controlling for age, sex, household wealth, education and ethnicity, higher cortisol was found to be significantly associated with poorer cognitive performance. Overall, the authors conclude that the study found a dose response relationship between cortisol and cognition, with higher cortisol levels being associated with poorer performance in a variety of cognitive domains throughout the neocortex and in the hippocampus (Lee et al., 2007). The KORA-Age study took a population based sample of 733 participants in Germany over the age of 64. Participants underwent a telephone-administered cognitive test and were then categorised into those with normal cognition (n=101), those with MCI (n=101) and those with dementia (n=33). Three salivary cortisol levels were measured for each participant; one on awakening, one 30 minutes after awakening and one in the evening, prior to going to bed. Participants collected the samples themselves, following verbal and written instructions. The study found that cognitive impairment was associated with reduced morning to evening cortisol levels, however in multivariate analysis, when gender was stratified for, the relationship was significant in men (n=375), but not women (n= 358). One flaw of this study is that cognitive status was determined by telephone interview, which may be difficult for those with hearing impairment, and may therefore give an overestimate of cognitive impairment. The third population based study (Schrijvers et al., 2011) (a part of the Rotterdam Study), involved

participants (n=3341) who were free from dementia at baseline. It did not find any association between baseline morning serum cortisol levels and subsequent development of dementia, however neither diurnal cortisol measurements nor serial cortisol measurements were taken.

Cortisol in cerebrospinal fluid has been shown to be associated with dementia, with cortisol levels correlating negatively with cognitive test scores (Gil-Bea et al., 2010), and an association between the ApoE genotype (associated with Alzheimer's dementia) and higher cortisol levels has also been demonstrated (Peskind et al., 2001). No such association was found between cortisol levels and MCI (Gil-Bea et al., 2010, Popp et al., 2015), suggesting that perhaps HPA axis dysregulation (resulting in elevated cortisol levels) is a consequence of dementia, or that dysregulation does not become detectable until dementia has developed. However, the diagnostic criteria used for MCI tend to vary between studies, and it may simply be that the diagnostic criteria are not robust enough, meaning that the overall picture is clouded by diagnostic uncertainty (Pendlebury et al., 2013).

There are no previous studies of the relationship between cortisol levels and cognitive outcome specifically after stroke. As discussed in chapter 2 , cortisol levels are elevated after acute stroke (for at least seven days) and higher cortisol levels after stroke have been found to be associated with higher dependency levels, longer length of stay, morbidity (such as depression) and mortality. As discussed in chapter 5, it is plausible that dysregulation of the HPA axis after stroke may be implicated in the pathogenesis of post-stroke cognitive impairment and dementia. Basal levels of cortisol are known to enhance memory formation, but stress levels supress this function (Chapman and Seckl, 2008). Furthermore, stress levels of cortisol are known to reduce neuronal survival following insults, such as stroke. The hippocampus, which plays a crucial part in several domains of memory, such as verbal memory, is particularly vulnerable to the deleterious effects of cortisol (Chapman and Seckl, 2008). As discussed in chapter 1, section 1.4.3, delirium may affect the HPA axis and cognition after stroke (although the direction of causation cannot be inferred from these studies) and so it is also important to take into account the possible effect of delirium on cortisol levels and on cognition in this study.

In summary, high cortisol levels have been shown to be associated with poorer cognitive function in the general older adult population in two out of three large studies, and CSF cortisol has been shown to be elevated in dementia. The relationship between cortisol and

MCI is less clear, with no strong association being found, however this must be interpreted with caution, as the definition of MCI has been heterogeneous amongst studies to date. It is plausible that cortisol may be associated with cognitive function after stroke, but no studies to date have investigated this association.

The aim of this study was to investigate the relationship between salivary cortisol levels after stroke, delirium and cognitive function in the 1 year after stroke.

The hypothesis addressed in this chapter is: Dysregulation of the HPA axis in the first week after stroke is associated with cognitive decline at up to 12 months, when the effect of delirium is controlled for.

7.2 Methods

7.2.1 Recruitment

Participants with acute stroke, aged over 60, were recruited consecutively from the acute stroke unit at the Royal Infirmary of Edinburgh, by me (Geriatric Medicine Registrar (AJB)), between October 2012 and March 2014, as described in detail in chapter 4. Exclusion criteria were: diagnosis of subarachnoid haemorrhage or TIA, tumour or other stroke mimic seen on imaging, current or recent use (within 6 months) of oral or inhaled corticosteroids, active alcohol withdrawal and inability to speak English prior to stroke. Informed consent was obtained from those deemed to have capacity (as assessed by AJB) or proxy consent was obtained from those who lacked capacity to provide consent. Ethical approval was obtained for the study from the Scotland A Research Ethics committee. The NHISS score and APACHE II scores were completed at baseline, as a measure of stroke severity and current illness burden respectively. These are described in more detail in section 4.3.

7.2.2 Delirium assessment

Participants were assessed for the presence of delirium, based on DSM IV criteria, on alternate days during the first week after stroke onset and then on days 14, 28, and at 4 months and 12 months. A detailed description of the protocol for delirium assessment is given in section 4.4.

7.2.3 Cognitive assessment

Participants completed the Montreal Cognitive Assessment (MoCA, to assess global cognition), National Adult Reading Test (to assess pre-morbid IQ, see section 4.6.1) and Digit Span Forwards and Backwards (to assess attention and working memory) at baseline, or as soon as practically possible after recruitment if illness precluded completion at baseline (all were completed within 2 weeks of recruitment). An informant (usually the nearest relative) completed the IQCODE at baseline, to screen for undiagnosed pre-existing cognitive impairment (those with an IQCODE score of <3.19 were deemed to have normal cognition (prior to stroke onset), those with a score of ≥3.19-3.43 were deemed to have evidence of pre-existing mild cognitive impairment and those with a score of ≥3.44 were deemed to have evidence of pre-existing dementia (Harwood et al., 1997, Li et al., 2012)). At 1 month, 4

months and 12 months post-stroke participants underwent more detailed neuropsychiatric testing. This consisted of a cognitive test battery comprising the MoCA, Digit Span Forwards and Backwards, Verbal Fluency (FAS and animals), Visual Reproduction, List Learning, Victoria Stroop Test and Digit Symbol Substitution Test. The tests and testing protocol are described in detail in section 4.6.

7.2.4 Salivary cortisol sample collection

Saliva was collected the morning (0930) and afternoon (1530) at each assessment. Thus samples were collected on the day of recruitment, and then on alternate days for the first week after stroke, and then at day 7 and day 14, 1 month, 4 months and 12 months after stroke onset. The collection process and devices are described in detail in section 4.8.

7.2.5 Salivary cortisol assays

Salivary cortisol was measured using an Enzyme-Linked Immunosorbant Assay (ELISA). This was performed by The Dresden LabService Gmbh (Tatzberg 47-49, 01307, Dresden, Germany)

7.2.6 Statistical Analysis

Group comparisons were made to compare cortisol levels between those who had delirium (either in isolation, or on a background of MCI or dementia), dementia only and those who were cognitively normal, using the Kruskal-Wallis test. Spearman's correlations between cortisol levels and cognitive test scores were then calculated. Dr Daniel Davis performed mixed effects multiple regression modelling, using the statistical package Stata 13.1, to examine the effects of cortisol and delirium on global cognitive function (measured by the MoCA) at baseline and over the 12 month follow-up period. Longitudinal change in cognitive outcome was estimated using random-effects models, where individuals were allowed to have random intercepts (cognitive score at first measurement) and random slopes (rate of change in cognition). The time metric was "time in study" and only linear relationships were considered. Covariance matrices were unstructured. The following covariates were considered for the intercept and slope: age, sex, NIHSS score on admission, IQCODE score, Charlson Comorbidities Index score and APACHE II score. Model fit was assessed using maximum likelihood estimates. Assumptions were checked by constructing Q–Q plots of the standardised residuals.

7.3 Results

95 participants were recruited from the acute stroke unit at the Royal Infirmary of Edinburgh, as described in detail in chapter 4. Baseline data are reported in chapter 5 (table 5.1).

7.3.1 Cortisol levels and their relationship with delirium and dementia

Group comparisons were made to compare salivary cortisol levels between those who developed delirium (with or without underlying dementia or MCI), those who had MCI/dementia only, and those who had neither delirium nor dementia (table 7.1). There were no significant differences between any of the three groups' cortisol levels at any of the time points. Differences between the groups' cortisol levels approached significance at the 4 month (am) time point. At this time point those with delirium on MCI/dementia had the highest cortisol levels, and the other 2 groups had similar levels.

Table 7.1 Group comparisons of median salivary cortisol levels at baseline, 1 month, 4 months and 12 months after stroke onset, based on delirium and MCI/dementia status (those with delirium defined as delirium diagnosed ever during the course of the study)

Time point	Median salivary cortisol in nmol/L (IQR)			p◊
	Delirium and delirium on MCI/dementia only	MCI/Dementia only	Neither delirium nor dementia	
Baseline (days 0-7) AM	27.0 (20.2-31.0) n=23	23.0 (13.5-31.2) n=26	23.2 (17.2-31.9) n=38	0.39
Baseline (days 0-7) PM	17.9 (11.7-26.1) n=20	13.6 (6.6-19.0) n=27	13.2 (9.4-17.8) n=38	0.24
Baseline ratio AM:PM	1.31 (0.95-1.82) n=19	1.8 (1.2-2.2) n=25	1.7 (1.2-2.5) n=36	0.40
1 month AM	31.3 (16.5-39.1) n=18	21.5 (17.6-34.3) n=23	22.5 (18.8-29.4) n=35	0.19
1 month PM	16.4 (11.1-22.5) n=14	12.9 (8.9-15.3) n=22	12.1 (9.1-16.0) n=37	0.18
1 month ratio AM:PM	1.7 (1.4-2.0) n=11	2.1 (1.4-2.9) n=19	1.9 (1.3-3.0) n=34	0.83
4 months AM	29.8 (18.7-34.2) n=15	21.6 (17.1-26.2) n=20	22.4 (14.4-30.0) n=41	0.04
4 months PM	14.5 (8.9-19.1) n=14	12.4 (7.5-15.7) n=24	12.5 (7.9-16.3) n=35	0.27
4 months ratio AM:PM	2.0 (1.4-2.0) n=11	2.1 (1.0-2.7) n=19	1.8 (1.2-2.8) n=29	0.96
12 months AM	23.6 (18.2-36.5) n=15	23.3 (15.1-37.4) n=24	25.8 (20.1-37.6) n=28	0.74
12 months PM	13.9 (7.9-22.6) n=14	11.4 (6.8-27.3) n=24	10.0 (5.4-13.7) n=28	0.29
12 months AM:PM	1.9 (1.3-3.3) n=13	1.4 (1.0-3.1) n=23	3.1 (1.6-4.1) n=26	0.16

◊Kruskal-Wallis test

7.3.2 Correlations between cortisol levels and cognitive test scores

As the data are non-parametric, Spearman's correlations were used to examine the relationships between median cortisol levels in the first 7 days after stroke and cognitive function at baseline (table 7.2) and also to examine the relationship between peak cortisol levels in the first 14 days after stroke and cognitive function at baseline (table 7.3). Median week one cortisol levels did not correlate with any of the cognitive test scores. Peak afternoon cortisol levels correlated with the baseline MoCA score (with a higher cortisol levels correlating with a lower MoCA score), but not with any other scores, and peak morning and the peak ratio cortisol levels didn't correlate with any of the cognitive test scores.
At 1 month follow-up (table 7.4), the ratio of morning to afternoon cortisol correlated with MoCA and list learning scores (total recall and delayed recall), with a higher ratio (indicating maintenance of the normal circadian rhythm) being associated with higher scores. No significant correlation between cortisol levels and cognitive test scores were found at 4 months and 12 months after stroke (table 7.4).

Table 7.2 Correlations between median morning, median afternoon and median ratio (morning: afternoon) cortisol levels during the first week after stroke and baseline cognitive test scores

Cognitive test	Median morning cortisol level (days 0-7) Rho, p value	Median afternoon cortisol level (days 0-7) Rho, p value	Median ratio of morning to afternoon peak cortisol level (days 0-7) Rho, p value
NART	ρ=0.218 p=0.057	ρ=0.086 p=0.453	ρ=0.209 p=0.076
MoCA	ρ=-0.140 p=0.200	ρ=-0.012 p=0.921	ρ=0.044 p=0.725
DSST	ρ=0.059 p=0.710	ρ=0.055 p=0.721	ρ=0.105 p=0.508
List learning recall	ρ=0.007 p=0.957	ρ=0.005 p=0.968	ρ=-0.016 p=0.900
List learning delayed recall	ρ=0.010 p=0.935	ρ=0.134 p=0.275	ρ=-0.031 p=0.810
List learning retention	ρ=0.051 p=0.685	ρ=0.172 p=0.163	ρ=-0.046 p=0.720
Verbal fluency	ρ=-0.460 p=0.691	ρ=-0.009 p=0.937	ρ=0.010 p=0.935
Visual reproduction	ρ=0.112 p=0.445	ρ=0.182 p=0.210	ρ=-0.067 p=0.656
Digit span (forwards)	ρ=0.017 p=0.884	ρ=-0.097 p=0.430	ρ=0.059 p=0.625
Digit span (backwards)	ρ=0.025 p=0.825	ρ=-0.057 p=0.621	ρ=0.132 p=0.267

ρ= Spearman's Rho correlation coefficient

Table 7.3 Correlations between peak morning, peak afternoon and peak ratio (morning: afternoon) cortisol levels during the first 14 days after stroke and baseline cognitive test scores

Cognitive test	Peak morning cortisol level (days 0-14) Rho, p value	Peak afternoon cortisol level (days 0-14) Rho, p value	Ratio of morning to afternoon peak cortisol level Rho, p value
NART	ρ=0.119 p=0.289	ρ=-0.033 p=0.772	ρ=0.162 p=0.154
MoCA	ρ=-0.146 p=0.170	ρ=**-0.318** **p=0.003****	ρ=0.192 p=0.079
DSST	ρ=0.107 p=0.471	ρ=-0.51 p=0.728	ρ=0.101 p=0.493
List learning recall	ρ=-0.170 p=0.886	ρ=-0.112 p=0.352	ρ=-0.001 p=0.994
List learning delayed recall	ρ=0.058 p=0.631	ρ=-0.057 p=0.637	ρ=0.045 p=0.712
List learning retention	ρ=0.135 p=0.267	ρ=-0.007 p=0.951	ρ=0.103 p=0.401
Verbal fluency	ρ=-0.010 p=0.931	ρ=-0.103 p=0.367	ρ=0.119 p=0.302
Visual reproduction	ρ=0.052 p=0.718	ρ=0.137 p=0.331	ρ=-0.226 p=0.111
Digit span (forwards)	ρ=-0.152 p=0.169	ρ=-0.007 p=0.949	ρ=-0.171 p=0.133
Digit span (backwards)	ρ=-0.079 p=0.479	ρ=-0.112 p=0.321	ρ=0.010 p=0.927

ρ= Spearman's Rho correlation coefficient

Table 7.4 Correlations between contemporaneous cognitive test scores and cortisol levels, at 1 month, 4 months and 12 months after stroke

Cognitive test	1 month cortisol level Rho, p value			4 months cortisol level Rho, p value			12 months cortisol level Rho, p value		
	AM	PM	Ratio (am:pm)	AM	PM	Ratio (am:pm)	AM	PM	Ratio (am:pm)
MoCA	ρ=0.097 p=0.426	ρ=-0.210 p=0.074	ρ=0.272 p=0.030	ρ=-0.119 p=0.350	ρ=-0.046 p=0.701	ρ=0.051 p=0.707	ρ=0.092 p=0.460	ρ=-0.009 p=0.942	ρ=0.035 p=0.787
DSST	ρ=0.017 p=0.914	ρ=-0.065 p=0.684	ρ=-0.048 p=0.776	ρ=-0.178 p=0.248	ρ=-0.054 p=0.699	ρ=-0.167 p=0.284	ρ=-0.066 p=0.970	ρ=-0.041 p=0.774	ρ=0.023 p=0.875
List learning recall	ρ=0.118 p=0.353	ρ=-0.154 p=0.237	ρ=0.269 p=0.049	ρ=-0.134 p=0.331	ρ=-0.066 p=0.595	ρ=0.016 p=0.908	ρ=0.182 p=0.154	ρ=0.122 p=0.341	ρ=0.010 p=0.938
List learning delayed recall	ρ=0.180 p=0.154	ρ=-0.151 p=0.245	ρ=0.332 p=0.014	ρ=-0.122 p=0.376	ρ=-0.072 p=0.566	ρ=0.001 p=0.996	ρ=0.092 p=0.472	ρ=0.096 p=0.454	ρ=-0.046 p=0.726
List learning retention	ρ=0.162 p=0.205	ρ=-0.086 p=0.508	ρ=0.266 p=0.052	ρ=0.036 p=0.788	ρ=-0.021 p=0.865	ρ=0.024 p=0.864	ρ=0.030 p=0.815	ρ=0.022 p=0.867	ρ=-0.019 p=0.886
Verbal fluency	ρ=0.102 p=0.394	ρ =0.188 p=0.120	ρ=-0.074 p=0.569	ρ=-0.105 p=0.423	ρ=0.035 p=0.774	ρ=-0.045 p=0.744	ρ=0.129 p=0.307	ρ=0.046 p=0.715	ρ=0.005 p=0.968
Visual reproduction	ρ=0.030 p=0.845	ρ=-0.197 p=0.194	ρ=0.209 p=0.201	ρ=0.019 p=0.903	ρ=0.015 p=0.917	ρ=-0.069 p=0.654	ρ=0.110 p=0.422	ρ=-0.108 p=0.438	ρ=0.146 p=0.296

Digit span (forwards)	ρ=-0.76 p=0.518	ρ=-0.200 p=0.869	ρ=-0.047 p=0.717	ρ=-0.080 p=0.540	ρ=0.059 p=0.627	ρ=-0.056 p=0.684	ρ=0.167 p=0.179	ρ=-0.096 p=0.445	ρ=0.214 p=0.094
Digit span (backwards)	ρ = -0.44 p=0.713	ρ=0.059 p=0.623	ρ=-0.016 p=0.903	ρ=-0.154 p=0.235	ρ=0.083 p=0.497	ρ=-0.115 p=0.403	ρ=0.010 p=0.936	ρ=-0.141 p=0.260	ρ=0.177 p=0.168

ρ= Spearman's Rho correlation coefficient

Table 7.4 (continued) Correlations between contemporaneous cognitive test scores and cortisol levels, at 1 month, 4 months and 12 months after stroke

7.3.3 Trajectory of global cognition over 12 months and its relationship to salivary cortisol levels and delirium

As discussed in chapter 6, there was an overall trend for improvement in the MoCA score (as a measure of global cognition) in the whole cohort over the 12 month follow-up period. Mixed effects multiple regression models were used to examine the effect of salivary cortisol (median at baseline, days 0-7) on MoCA scores at baseline after stroke (table 7.5). This shows that neither morning nor afternoon median cortisol levels at baseline nor the morning: afternoon ratio of cortisol at baseline are associated with MoCA scores at baseline. Mixed effects multiple regression models were then used to investigate the effects of median baseline cortisol levels (days 0-7) on the rate of change of the MoCA over 1 year (MoCA trajectory) (table 7.5). This showed that neither morning nor afternoon median salivary cortisol levels, nor the ratio of morning to afternoon cortisol levels significantly affected the rate of change of the MoCA score over 1 year.

Mixed effects multiple regression models were then used to investigate the effect of cortisol and delirium on the baseline MoCA score (table 7.6). This showed that when adjusted for the presence of delirium, only the median morning: afternoon ratio of cortisol was associated with the baseline MoCA. Finally, mixed effects multiple regression models were used to investigate the effects of cortisol and delirium on the rate of change of the MoCA score over 1 year (table 7.7). This showed that when adjusted for the presence of delirium, cortisol was not associated with the rate of change of the MoCA score over 1 year.

Table 7.5 Mixed effects multiple regression modelling investigating the effect of baseline salivary cortisol (median, days 0-7) on MoCA scores at baseline and on the MoCA trajectory over 12 months.

Variable	Coefficient (MoCA score at intercept)	95% CI	p
Median morning cortisol levels at baseline (nmol/L)-effect on baseline MoCA	-0.003	-0.010-0.005	0.50
Median afternoon cortisol levels at baseline (nmol/L)-effect on baseline MoCA	-0.001	-0.001-0.0001	0.11
Median morning: afternoon cortisol levels at baseline (nmol/L)-effect on baseline MoCA	0.216	-0.02-0.451	0.07
Median morning cortisol levels at baseline (nmol/L)-effect on MoCA trajectory	$2x10^{-5}$	$-4x10^{-5}$-$8x10^{-5}$	0.57
Median afternoon cortisol levels at baseline (nmol/L)-effect on MoCA trajectory	$1x10^{-5}$	$-4x10^{-5}$-$2x10^{-5}$	0.58
Median morning: afternoon cortisol levels at baseline (nmol/L)-effect on MoCA trajectory	$3x10^{-4}$	$-5x10^{-4}$ -$1x10^{-3}$	0.46

Table 7.6 Mixed effects multiple regression model-investigating the relative effects of baseline salivary cortisol (median, days 0-7) and delirium on MoCA scores at baseline.

Variable	Coefficient (change in MoCA score per day)	95% CI	p
Median morning cortisol levels	-0.002	-0.009-0.005	0.58
Median afternoon cortisol levels	-0.0004	-0.001-0.0003	0.28
Median morning: afternoon cortisol levels	0.253	0.019-0.488	0.03
Delirium (present at any point in the 12 months)	-5.60	-7.32- -3.87	<0.001

Table 7.7 Mixed effects multiple regression model-investigating the relative effects of baseline salivary cortisol (median, days 0-7) and delirium on the MoCA trajectory over 12 months.

Variable	Coefficient (change in MoCA score per day)	95% CI	p
Median morning: afternoon cortisol levels	0.313	-0.828-1.455	0.59
Delirium (present at any point in the 12 months)	-11.40	-14.89- -7.92	<0.001

7.3.4 Trajectory of neuropsychological test battery over 12 months and its relationship to salivary cortisol levels and delirium

Mixed effects multiple regression models were used to investigate the effect of delirium and salivary cortisol levels (median, days 0-7) on the trajectory of the neuropsychological tests (excluding the MoCA) in the 12 months after stroke (table 7.8). This shows that when adjusted for the effects of delirium, salivary cortisol levels were not associated with the rate of change in the test score over the 12 months.

Table 7.8 Mixed effects multiple regression models-investigating the effects of baseline salivary cortisol (median, days 0-7) and delirium on the trajectory of each neuropsychological test over 12 months. (Models were built separately for each neuropsychological test)

Variable	Coefficient (change in score per day)	95% CI	p
Digit span	$1.25x10^{-5}$	-0.002-0.002	0.99
Verbal fluency	$-1.1x10^{-5}$	-0.003-0.003	0.99
HVLT-R List Learning	-0.001	-0.003-0.0004	0.12
Visual reproduction	-0.006	-0.024-0.012	0.52
Victoria Stroop	0.0002	-0.0002-0.0006	0.34
DSST	0.003	-0.003-0.009	0.39

7.4 Discussion

This study found that there were no significant differences in salivary cortisol levels between those who developed delirium (with or without pre-existing dementia), those who had dementia only and those who had neither delirium nor dementia. Median cortisol levels in the first week for the whole cohort did not correlate with any of the cognitive test scores. Peak afternoon cortisol levels (in the first 14 days after stroke) significantly correlated with baseline MoCA scores, but not with any of the other cognitive tests, and neither morning peak levels nor the peak morning to afternoon ratio correlated with any of the cognitive test scores. At 1 month follow-up neither morning nor afternoon cortisol levels correlated with cognitive test scores, but the ratio of morning to afternoon cortisol correlated with the MoCA score and the list learning recall and delayed recall scores. At 4 months and 12 months cortisol levels did not correlate significantly with any of the cognitive tests. In mixed effect multiple regression analysis, no association between baseline cortisol levels and baseline MoCA scores was found. When adjusted for the presence of delirium, the median morning: afternoon ratio of cortisol during week 1 after stroke was significantly associated with the baseline MoCA score (table 6). A larger ratio (meaning preservation of the normal diurnal pattern) was associated with a higher MoCA score, and so conversely loss of the normal diurnal pattern (indicated by a lower morning to afternoon ratio) was associated with a lower MoCA score There was no significant association between cortisol levels during week 1 after stroke and the rate of change of the MoCA nor the neuropsychological test battery scores in the 12 months after stroke.

This provides the first evidence of a possible relationship between the HPA axis and cognitive decline after stroke. The association between baseline global cognition (measured at the first assessment after stroke onset) and salivary cortisol is intriguing. It is interesting that no association was found between cortisol and the trajectory of cognitive function in the year after stroke, implying that if the association is real, the effects of cortisol on cognition are greatest in the first few days after stroke. The effect of delirium may confound this finding, as although the presence of delirium was controlled for in the statistical model, it is possible that a brief episode of

delirium, or sub-syndromal delirium (whereby some of the features of delirium are present, but the full diagnostic criteria are not met) may have been missed, but that this may have had a detrimental effect on the baseline MoCA score. Additionally, there may also be short-term effects on cognition (and indeed on cortisol levels) of the stroke itself and any other intercurrent illness present at the time of assessment. It is also interesting that in this small study it was the ratio (morning: afternoon), but not absolute values of cortisol that were associated with cognition. This implies that it may be the loss of diurnal rhythm, resulting in higher circulating levels of cortisol in the afternoon and evening, which is associated with cognitive decline. This finding replicates those of the population based KORA-age study (Johar et al., 2015) (described in the introduction), which found a loss of diurnal rhythm in cortisol was associated with cognitive decline (in men but not in women, although there were relatively few women in the cognitively impaired group). A second population based study (of 914 non-demented participants) from Amsterdam by Gerritsen and colleagues (Gerritsen et al., 2011) also found a loss of diurnal rhythm in association with cognitive decline, but only in those who were APOE-ε4 allele carriers. This study also found that higher evening cortisol levels were associated with a more rapid decline in delayed recall, which is thought to be a hippocampal dependant memory function. It is important to note that neither of the aforementioned studies included participants after stroke per se, and neither are able to infer the direction of causation in terms of whether loss of a diurnal cortisol rhythm is a cause or a consequence of cognitive decline.

The finding of an association between a loss of diurnal cortisol rhythm and cognitive decline in those who have recently had a stroke is important as it is often assumed that the pathological processes underlying cognitive decline after stroke are predominantly vascular. The findings of this study suggest that cortisol may also play a role, perhaps by deleterious actions on neurones (particularly in the hippocampus). If this association is replicated in larger prospective studies (which would be required in order to infer the direction of causality) it would offer intriguing targets for therapeutic intervention.

There are some limitations to this study which should be acknowledged. The study findings are confounded by the fact that there was significant attrition of participants

from the delirium group in particular, who are more likely to have underlying cognitive impairment. This means that those who underwent cognitive testing at 4 months and 12 months were generally physically and cognitively more robust. There is a large amount of missing data, particularly from the cognitive tests and again the frailer participants were more likely to ask to terminate testing early and hence have data missing from their cognitive profile. Cortisol samples were taken in the morning and afternoon for reasons of practicality. It could be argued that samples should have been taken earlier in the morning and later in the evening to give a clearer indication of the diurnal variation, however this would have posed logistical problems and may have reduced the number of samples collected. Although every effort was made to ascertain whether participants had been free from delirium in the time between hospital discharge and follow-up visits, it is possible that brief new episodes may have been missed. It was also difficult to establish the duration of a new episode of delirium which had occurred in the community, particularly if it was brief and did not lead to hospital admission (as was the case for one participant). These factors mean that the true incidence and duration of delirium and its impact on post-stroke cognitive trajectory may have been underestimated. Finally, as discussed in section 5.4, caution is required in the interpretation of the multivariate models presented in this chapter, as although the number of predictors in the model did not exceed 1 per 10 participants, the effect size of cortisol on cognition and delirium after stroke was not known, and therefore not taken into account. Once again, as described in section 5.4, a Bonferroni correction may be applied in order to account for multiple comparisons, however again the main findings presented in this chapter were not altered when this correction was applied to the analysis.

In summary, this study has shown that median cortisol levels in the first week after stroke are associated with the presence of delirium and, after adjusting for the presence of delirium, the median morning: afternoon ratio of cortisol during week 1 after stroke was significantly associated with the baseline MoCA score. There was no significant association between cortisol levels in the first week after stroke and the trajectory of global cognition (measured by the MoCA) over 12 months, in the subset of participants who completed the full 12 months of the study.

Chapter 8: Delirium after stroke and neuroimaging biomarkers on Computed Tomography (CT) scans

8.1 Introduction

In conditions other than stroke, neuroimaging studies have found a possible link between delirium and the presence of white matter lesions (WMLs), however the studies are small and have methodological weaknesses, such as failing to control, for age (Soiza et al., 2008), or assessing for delirium retrospectively (Hatano, 2013). A recent, more methodologically sound, study of 146 participants without dementia undergoing elective surgery, found no association between brain atrophy, WMLs (detected using MRI) and delirium incidence or severity (Cavallari, 2015).

Almost all patients with a suspected stroke admitted to hospital in developed countries will have a CT brain scan soon after admission (within 24 hours of admission in the United Kingdom). Whilst some patients may go on to have an MRI brain scan as well, many patients will not be able to tolerate this because of the time required to complete the scan and also because some find that the MRI scanner is noisy or makes them feel claustrophobic. Furthermore, up to one fifth of patients are not able to undergo MRI scanning either because they are too unwell or because they have an intracerebral or intraocular metallic foreign body or a pacemaker (Wardlaw, 2004). Computed Tomography, whilst providing a less detailed scan, is much quicker to complete (usually completed from beginning to end within ten minutes) and suitable for nearly all, and so studies using CT closely reflect the whole clinical population. Furthermore, whilst CT is primarily performed from a clinical point of view to exclude haemorrhage, early signs of ischaemic stroke can also be seen on CT, such as swelling, hypodensity and hyperdense vessels, as well as any old stroke lesions. The presence and severity of white matter lesions (WMLs) and any cerebral atrophy can also be determined from CT brain scans. Indeed, for some parameters such as global cerebral atrophy and presence of moderate-severe white matter lesions CT and MRI measures show very good agreement (Wattjes M.P, 2009, Wahlund, 2001).

As discussed in section 1.5.5 few studies have investigated the relationship between neuroimaging biomarkers, such as brain atrophy and white matter lesions, and delirium after stroke. To date, just two published studies have investigated this. Henon and colleagues recruited 202 participants, of whom 120 had a computed tomography (CT) scan and 82 had a Magnetic Resonance Imaging (MRI) scan. They found that those with delirium had more cortical atrophy , though there was no correction for age (Henon et al., 1999). Oldenbeuving and colleagues reported that in 527 stroke participants, brain atrophy on CT scan was associated with an increased risk of delirium in the first week after stroke. However, participants were assessed for just one week after stroke (or up to 15 days if they were diagnosed with delirium in the first week), meaning that delirium occurring after day 7 of stroke onset would be missed (Oldenbeuving, 2011).

The stroke lesion itself is known to influence outcome in terms of morbidity and mortality, for example haemorrhagic stroke confers a higher risk of mortality than ischaemic stroke (even after correcting for age and stroke severity)(Anderson, 2009). Again, as previously discussed, conflicting results have been found in terms of the types of acute stroke lesions which confer the highest risk of delirium, with Gustafson and colleagues finding that haemorrhagic stroke and left sided lesions conferred the highest risk, whilst Oldenbeuving and colleagues found an association with right hemisphere lesions (Henon et al., 1999, Oldenbeuving, 2011). To my knowledge no previous studies have investigated the relationship between old stroke lesions seen on neuroimaging and delirium after stroke.

This aims of this section of the study are to:

1. Analyse the relationship between post-stroke delirium and brain atrophy
2. Analyse the relationship between post-stroke delirium and WMLs
3. Analyse the relationship between post-stoke delirium and acute and previous stroke lesions

The main hypothesis addressed in this chapter is: Baseline pathology detected on admission CT brain scans, namely global brain atrophy, white matter lesions and a visible stroke lesion, are associated with the development of delirium after stroke.

8.2 Methodology

8.2.1 Imaging

All 95 participants recruited to the longitudinal cohort study of delirium and long-term cognitive impairment after stroke underwent CT brain scanning as part of their routine clinical care, prior to recruitment to the study, after stroke. Subjects were scanned in a Toshiba 64-slice or 128-slice scanner (the 64-slice scanner being replaced by a new 128-slice scanner during the study period). Brain scans were stored in the clinical Patient Archiving and Communication System (PACS). The study protocol, including how delirium was diagnosed, are described in detail in Chapter Two.

8.2.2 Data extraction

I (AJB), a Geriatric Medicine Registrar, underwent training with an experienced neuroradiologist (Dr Andrew J Farrall, AJF), on how to read and extract data from CT brain scans. This training consisted of a face to face briefing on how to use the data extraction tool and practical demonstrations, face to face, of how to analyse the scans in a systematic way. I also participated in the Acute CT Cerebral Evaluation of Stroke Study (ACCESS) as part of my training (www.neuroimage.co.uk), which involved interpreting scans and comparing responses against those of experts. A standard method of extracting information on stroke lesion location, anterior and posterior white matter lesions (using the Van Swieten scale (van Swieten et al., 1990)) and global cerebral atrophy (Pasquini et al., 2007), which has been successfully used in a previous study of fatigue after stroke (Kutlubaev et al., 2013) and also in a study of thrombolytic treatment after stroke (Wardlaw JM et al., 2015), was used for this study (see appendix 6) . Following recruitment to the study, but prior to delirium and cognitive assessments, I rated CTs using the standardised proforma previously described. AJF subsequently rated all scans, in batches of ten, blind to all other data. Scans were read by both me and AJF so that an inter-rater reliability analysis could be performed. With the exception of the inter-rater reliability, all results presented are based on the data extracted solely by AJF.

8.2.3 Delirium diagnosis

Delirium was diagnosed as outlined in detail in section 1.2.5 and section 4.5

8.2.4 Statistical analysis

Due to the use of short ordinal scales and the non-normal distribution of some of the data non-parametric statistical analysis was used. The Mann- Whitney U test (for continuous data) or the chi-square test (for nominal categorical data) was used to investigate the relationship between delirium diagnosis and baseline characteristics.

Inter-rater reliability:

The agreement between the two observers (inter-rater reliability) was tested by calculation of weighted kappa values. Weighted kappa values were chosen because the categories are ordered (i.e. none, mild, moderate or severe for both WMLs and atrophy) which allows account to be taken of the magnitude of discrepancy between the two raters. A kappa value of <0.2 signifies a poor strength of agreement, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 good and 0.81- 1.00 very good (Altman, 1991).

Neuroimaging features and delirium:

Pearson's chi-square test was used to investigate the relationship between delirium diagnosis (at any time point in the 12 month study period) and presence of an acute ischaemic lesion, acute haemorrhage, presence of a hyperdense artery, global cerebral atrophy (rated none, mild, moderate or severe) and WMLs (rated none, mild moderate or severe) and presence of an old stroke lesion.

Multivariate analysis:

Binary logistic regression, using the enter method, was used to explore the independent contributions of each variable. Delirium (binary outcome, yes/no) was the dependent variable. Predictor variables selected *a priori*, based on the hypothesis and findings in previously published studies, were WMLs (linear scale where 0 = none, 1 = mild 2=moderate and 3=severe), atrophy (linear scale from 0-4 as described for WMLs), presence of an acute stroke lesion and presence of an old stroke lesion. Co-variates selected *a priori* were age, sex, NIHSS score at admission (as a measure of stroke severity) and IQCODE (as a measure of prior cognitive impairment).

8.3 Results

All 95 participants had a CT brain scan and were included in this sub-study. Participant baseline descriptive information is presented in section 5.3. Twenty-six participants (27%) were diagnosed with a delirious episode at some point during the 12 month study period.

An acute stroke lesion was present in 40 (42%) participants overall, of whom 32 (34%) had an ischaemic lesion and 8 (8.4%) had a primary haemorrhagic lesion (table 8.1). Old vascular lesions were seen in 39 (41%) participants, cerebral atrophy (of any degree ranging from mild to severe) was present in 89 (93.6%) participants and WMLs were present in 70 (73.6%) participants. The atrophy and WMLs were symmetric in the majority of participants (85% of those with atrophy and 85% of those with WMLs). Finally, only one participant had an entirely normal scan, without any acute lesions, cerebral atrophy or WMLs.

Table 8.1 Descriptive statistics of participants with and without delirium in the 12 months after stroke

Characteristic	Delirium (n=26),	No delirium (n=69),	p
Age, median (IQR)	83.5 (79-85.3)	74 (68.5-82)	<0.001
Length of stay, days, median (IQR)	26.5 (11-51.8)	10 (5.5-23)	0.002
	n(%)	**n(%)**	
Males	10 (37)	46 (67)	0.01
Thrombolysed	11 (42)	13 (19)	0.58
Short IQCODE score ≥3.4	15 (56)	18 (26)	0.004
Acute ischaemic lesion	10 (37)	22(32)	0.545
Acute haemorrhage or haemorrhagic transformation	4(14.8)	4(6)	0.134
Hyperdense artery present	2(7.4)	5(7.2)	0.825
Atrophy present Degree of atrophy: Severe Moderate Mild None Atrophy globally symmetric	26(100) 6 12 8 0 24 (92.3)	63(91.3) 6 27 30 6 52(82.5)	0.007
White matter lesions present Degree of WMLs Severe Moderate Mild None WMLs globally symmetric	21(80.7) 2 8 11 5 20(80)	49(71) 6 13 30 20 41(83.6)	0.215
Old vascular lesion present Classification: Old cortical infarct Old striatocapsular infarct Old lacunar infarct Old brainstem infarct	13(50) 7 - 3 3	26(37.6) 14 1 8 1 1	0.276

POC - Package of care.

8.3.1 Inter-rater reliability (between neuroradiologist and Geriatric Medicine registrar)

The weighted kappa score for the rating of cerebral atrophy was 0.41 (moderate agreement) and the weighted kappa score for the rating of white matter lesions was 0.61 (good agreement).

8.3.2 Neuroimaging features and delirium

Delirium diagnosis was associated with the presence of global cerebral atrophy (100% of those diagnosed with delirium had atrophy compared with 91% of those in the non-delirium group). There was no relationship between the diagnosis of delirium and the presence of a visible acute or chronic, ischaemic or haemorrhagic lesion, the presence of a hyperdense artery or the presence of WMLs.

8.3.3 Multivariate analysis

The assumptions for logistic regression including linearity (meaning that there is a linear relationship between continuous predictor variables and the logit of the outcome variable (in this instance delirium presence or absence)), independence of errors (meaning that each case of data is not related) and Multicollinearity (meaning that predictor variables are not highly correlated) were tested and met. In terms of multicollinearity, all of the tolerance values are greater than 0.1 and Variance Inflation Factor (VIF) values are all less than 10, which are within recommended limits (Field, 2009), and analysis of the eigenvalues does not suggest collinearity between variables. A logistic regression (using the enter method) was performed with delirium (binary outcome, yes/no) as the dependent variable and WMLs (linear scale where 0 = none, 1 = mild 2= moderate and 3= severe), atrophy (linear scale from 0-4 as described for WMLs), presence of an acute stroke lesion, presence of a chronic stroke lesion, age, sex, NIHSS, and IQCODE as the predictor variables (table 8.2). A total of 95 cases were analysed and the model accounted for between 38% and 55% of the variance in delirium status. Table 3 shows the odds ratio and 95% confidence intervals (CI) for each of the predictor variables. This shows that only sex (being female), NIHSS score at baseline and presence of atrophy were independent predictors of delirium incidence. The model is corrected for all of the variables presented in the table.

Table 8.2 Results of the multivariate analysis; binary logistic regression model

Variable	Odds ratio	95% CI	p
Age (years)	1.07	0.98-1.18	0.114
Sex (female)	6.99	1.44-33.89	0.016
NIHSS	1.28	1.09-1.51	0.003
IQCODE score	6.23	0.36-107.84	0.208
Brain atrophy (0-3)	3.70	1.15-11.88	0.028
White matter lesions (0-3)	0.73	0.33-1.39	0.427
Visible acute stroke lesion	2.36	0.62-8.93	0.207
Visible old stroke lesion	0.494	0.13-1.90	0.304

8.4 Discussion

In this study of 95 participants, the relationship between post-stroke delirium and brain atrophy, WMLs and presence of acute and old stroke lesion visible on CT brain scans was investigated. The presence of brain atrophy was associated with delirium at up to 12 months after stroke , and with each increase in atrophy score of one point (on the scale where 0 = none, 1 = mild 2= moderate and 3= severe) participants were 3.36 times more likely to develop delirium. However, the presence of WMLs and visible acute or chronic stroke lesions were not associated with delirium. The severity of the stroke (measured by NIHSS at baseline) predicted delirium, with those with a higher score being more likely to develop delirium. Female sex, but not age, was also associated with delirium.

The finding that brain atrophy, but not WMLs, are associated with delirium after stroke is in keeping with findings of a previous study of delirium in the first week after stroke (Oldenbeuving, 2011). It is well established that the extent and rate of total brain atrophy and ventricular enlargement are associated with development of dementia, independent of hippocampal atrophy (Appleman, 2010). It is possible that those with pre-existing brain atrophy are more vulnerable to developing delirium, even if they do not have dementia, perhaps by virtue of the neuroanatomical changes found in atrophic brains such as narrowed gyri, widened sulci and enlarged ventricles, which in turn reflect neuronal loss (Gunther, 2012). Neuronal loss, perhaps in certain critical regions (Field, 2012), may reduce the resilience of the brain to resist acute dysfunction resulting from the precipitants commonly implicated in delirium.

Whilst I did not find a relationship between the presence of a visible stroke lesion and delirium, it is important to note that the baseline CT scans were all performed within 24 hours of stroke onset. It is possible therefore, for ischaemic, but not haemorrhagic strokes, that a subsequent scan may have revealed a stroke lesion, not evident on the initial scan and consequently an association between presence of an acute ischaemic stroke lesion and delirium could be missed. Thirty-seven out of 95 scans had a visible stroke lesion present, 13 out of 26 of those diagnosed with delirium had a stroke lesion visible on admission scan and 24 out of 69 of those not diagnosed with delirium had a visible lesion on the admission scan. The NIHSS score, as a marker of stroke severity, was associated with delirium. Again this is in keeping with the

findings of previous work (McManus, 2009, Oldenbeuving, 2011), however I assessed participants more frequently and over a longer period than these previous studies meaning that prolonged episodes of delirium or incident delirium occurring after the first few days of stroke was less likely to be missed . The finding of an association between female sex and delirium has not previously been reported, indeed neither female nor male sex was associated with delirium in the two previous studies of neuroimaging and stroke, and further research will be required to unpick whether this is a true association. It is interesting to note that age per se was not found to be associated with delirium in the multivariate analysis. In general, brain atrophy is associated with advancing age (Samorajski, 1976), however formal testing of covariance did not find a significant relationship which would preclude both being included in the statistical model. This may be because other factors, such as pre-existing dementia, were more important in determining which participants had an atrophied brain, rather than simply age in this cohort. However, several other well conducted studies which have not included brain atrophy in their analysis have tended to find an association between age and delirium after stroke (McManus, 2011, Henon et al., 1999, Edlund, 2006).

The inter-rater reliability between a neuroradiologist and geriatric medicine registrar was moderate for atrophy and good for WMLs. Few previous studies of inter-rater reliability for rating atrophy on CT scans have been conducted, although one study looking at the reliability between 4 neuroradiologist found fair agreement (kappa 0.24 (Leonardi, 1993)). This suggests that with appropriate training and the use of a validated tool, it is reasonable for a clinician to rate CT scans for atrophy, as inter-rater reliability is at least as good as that found between a group of experts.

This study has several strengths: I used data from routinely obtained clinical CT brain scans, which are available for all of the participants meaning that findings are more generalizable to the in-patient stroke population than if, for example, I had used research MRI scans for the study. It is not currently clear how well CT performs versus MRI in terms of detecting WMLs and atrophy on scans from stroke patients, although preliminary data suggests that there is good agreement between the scan data certainly in terms of detecting moderate to severe changes (Wattjes M.P, 2009, Wahlund, 2001). I recruited a sample of stroke participants with a median NIHSS score of 5 (range 1-28, IQR 4-8) which has been shown by a recent audit of UK

hospital admissions to be clinically representative in terms of stroke severity (the median NIHSS score for >11 000 admissions was 5, IQR 2-10)(Sentinal stroke national audit programme). I used a well validated assessment method to rate the CT parameters (Wardlaw JM et al., 2015), and used several well-validated methods to assess participants for the features of delirium, and assessments were carried out with sufficient regularity (and with the additional chart assessment tool and discussions with ward staff and family members), that it is unlikely that an episode of delirium was overlooked even though participants were not assessed face to face every day. It must be acknowledged, however, that a very mild or very brief episode which was neither detected by family members and clinical staff nor recalled by the patient could be overlooked. Particular attention was paid to the features of hypoactive delirium (for example reduced level of arousal) which may be overlooked by clinicians and family members if not specifically sought. Participants were followed-up for twelve months at intervals, as it is established that delirium may occur over a prolonged period following a stroke (McManus, 2009), previous studies have not assessed the whole cohort in this detailed way over a prolonged time period.

There are a number of limitations to this study: The study was relatively small (95 participants), however, in the field of delirium research few neuroimaging studies have been performed and even fewer have looked at neuroimaging and delirium after stroke (only two to my knowledge as previously discussed) . A systematic review in 2008 found that in the 12 included studies 5 to 69 participants with delirium were included and 11 to 197 controls (Soiza et al., 2008). Only 5% of participants in this study had a haemorrhagic stroke, whereas a recent audit found that around 10% of admissions to UK stroke units are due to haemorrhagic stroke (Sentinal stroke national audit programme), meaning that ischaemic stroke may be overrepresented in the study. There are a number of possible barriers to recruiting those who have suffered a haemorrhagic stroke, for example those with haemorrhagic strokes have a four-fold increase in the risk of death compared to those with ischaemic stroke (taking all ischaemic stroke types together) in the first few days after stroke, and on admission those with haemorrhagic stroke have higher stroke severity scores (Anderson, 2009). I controlled for undiagnosed dementia in the study using the IQCODE, which has been validated for use in this way (Jorm, 1995b), however it is important to acknowledge that the IQCODE relies on the informant recognising

changes in memory, prior to the stroke. To this end it is possible that some cases of pre-existing undiagnosed dementia may have been missed. As discussed in previous chapters, there was attrition of participants, particularly from the group who developed delirium. This may have had a bearing on the findings, as those with the most severe strokes (which are more likely to be visible lesions on CT scan) are more likely to have died early in their admission, meaning that delirium is perhaps less likely to have been diagnosed due to the short time frame of their admission. Finally, binary logistic regression analysis was performed on the data. Whilst this is an accepted method of analysing multivariate data with a binary outcome, it must be acknowledged that two of the predictor variables are ordinal (atrophy and WMLs), that is the values are ranked, in this case as none, mild, moderate and severe, but the real distance between each rank is unknown. In logistic regression, ordinal variables have to be treated either as nominal unordered categories or have to be numerical. In the former case information is lost as to the order of categories, and so I chose to label the categories numerically (0= none, 1= mild, 2= moderate and 3= severe). This assumes that one unit change, for example from 0 to 1 is approximately the same as one unit change from 2 to 3, an assumption which is difficult to verify in the context of brain atrophy.

In summary, I found that delirium at up to 12 months post-stroke was associated with cerebral atrophy on baseline CT brain. Baseline CT features may be useful, in conjunction with clinical measures, in aiding clinicians to predict who is particularly at risk of delirium. This may be particularly useful to predict those likely to develop hypoactive (or "quiet") delirium which is often under-recognised. Strategies to reduce the risk of delirium in the most vulnerable can then be considered and targeted appropriately. Moreover the results provide an intriguing insight into the potential pathophysiological mechanisms underpinning delirium after stroke, which require further investigation.

Chapter 9: Linear Computed Tomography (CT) Measurements and Delirium

9.1 Introduction

Brain atrophy identified on CT, in non-stroke patients, is known to be associated with delirium (Koponen, 1989). This association also held true in the cohort of stroke patients presented in this thesis (chapter 8). A recent study using MRI brain scans in non-stroke Intensive Care Unit (ICU) survivors suggested that atrophy in specific regions of the brain (for example superior frontal atrophy) may be associated with delirium duration, which in turn is associated with long-term cognitive impairment (Gunther, 2012). This is intriguing, and it is not clear if atrophy in specific regions of the brain is associated with delirium or indeed cognitive impairment after stroke. As discussed in chapter 8, MRI scanning is not suitable for a significant proportion of stroke patients and a reproducible method of assessing regional brain atrophy on CT scans may be beneficial for future studies of these associations.

The use of quantitative linear measurements of CT scans to assess the presence and degree of brain atrophy has been investigated as a tool to detect (n=129) (Jobst, 1992) and diagnose (n=307) (Zhang, 2008) Alzheimer's Disease (AD). This involves taking measurements of pre-defined features on CT scans using either callipers for hard copies of scans or computer graphical functions for electronic scans. Using these methods, atrophy of the medial temporal lobe has been shown to be associated with AD determined histopathologically, although the study controlled only for age, but not for other confounders such as previous strokes (Jobst, 1992). A second study found that temporal horn and supracellar cistern measurement made a useful contribution to making a diagnosis of AD, when combined with other clinical factors (such as apolipoprotein E4 genotype) (Zhang, 2008), however this methodology has not been widely adopted subsequently. The associations of quantitative CT measurements of brain atrophy with delirium, in general, but also in particular after stroke have not previously been investigated.

It is possible that quantitative analysis of CT scans may be more objective than so called 'qualitative' analysis (qualitative in this context meaning, for example, the use

of short-ordered atrophy rating scales, whereby atrophy is rated as none, mild , moderate or severe, by a trained observer) in clinical research and possibly also in clinical practice. Inter-observer reliability for qualitative atrophy rating scales has been shown to be only fair, even amongst expert neuroradiologists (Leonardi, 1993), and consequently subjective estimation of brain atrophy may not be sufficiently reliable if the presence or absence of atrophy is to contribute to research findings and clinical decision making.

This study was not part of the original study protocol and should be considered exploratory.

9.2 Aims

1. To investigate the intrarater and inter-rater reliability of linear measurements of CT brain scans
2. To investigate the relationship between brain atrophy determined by a qualitative rating scale and brain atrophy determined by quantitative linear measurements of CT brain scans in patients presenting after stroke.
3. To investigate the relationship between quantitative linear measurements of CT brain scans and delirium after stroke

9.3 Methodology

95 participants were recruited to the main longitudinal study and underwent baseline CT brain imaging as part of routine clinical care as previously described. Subjects were scanned in a Toshiba 64-slice or 128-slice scanner. Brain scans were stored in the clinical Picture Archiving and Communication System (PACS). The study protocol, including how delirium was diagnosed and how scans were analysed using a qualitative rating scale, are described in detail in chapter 8.

9.3.1 Data extraction

I (AJB) was trained by a Research Fellow (Dr Karen Ferguson) with extensive experience in both qualitative and quantitative brain imaging analysis. I was given verbal and written instructions on how to identify, align and measure CT parameters and was observed analysing several practice scans, prior to commencing the study. I then took linear measurements directly from electronic images stored on the PACS system for the participants in this study, blind to all other data about the participant. Images were aligned to the AC-PC line and adjusted for symmetry. The graphics function of PACS was then used to perform the measurements. Measurements were based on a scheme, published by Zhang and colleagues (Zhang, 2008). The measurements taken are shown in figure 9.1. Measurements A, B, C, H, I, O, P and N are measured on the slice where the feature is most clearly visualised, measurement E, F and J are measured on the slice that shows most prominently the heads of the caudate nuclei, G is measured on the slice displaying most of the body of the lateral ventricle and M is measured from the top slices without displaying the lateral ventricle. Measurements took approximately 20 minutes to complete for each scan. With the exception of those measurements relating to skull size (A, B and C), a larger measurement would be indicative of a greater degree of brain atrophy.
From these measurements, the following indices and ratios were calculated:
Evans ratio (E/C), bicaudate ratio (F/A), Huckman number (E+F), cella media index (G/A), third ventricular ratio (H/A), ventricle index (J/E), frontal subarachnoid ratio (I/B), four cortical sulcal ratio (M/A), cistern ambiens ratio (N/A) temporal horn ratio (O/A) and supracellar cistern ratio (P/A). Those ratio's which involve measurements A, B or C (measurements of the skull vault) are included as they correct for skull size (which is an indirect measure of peak brain size).

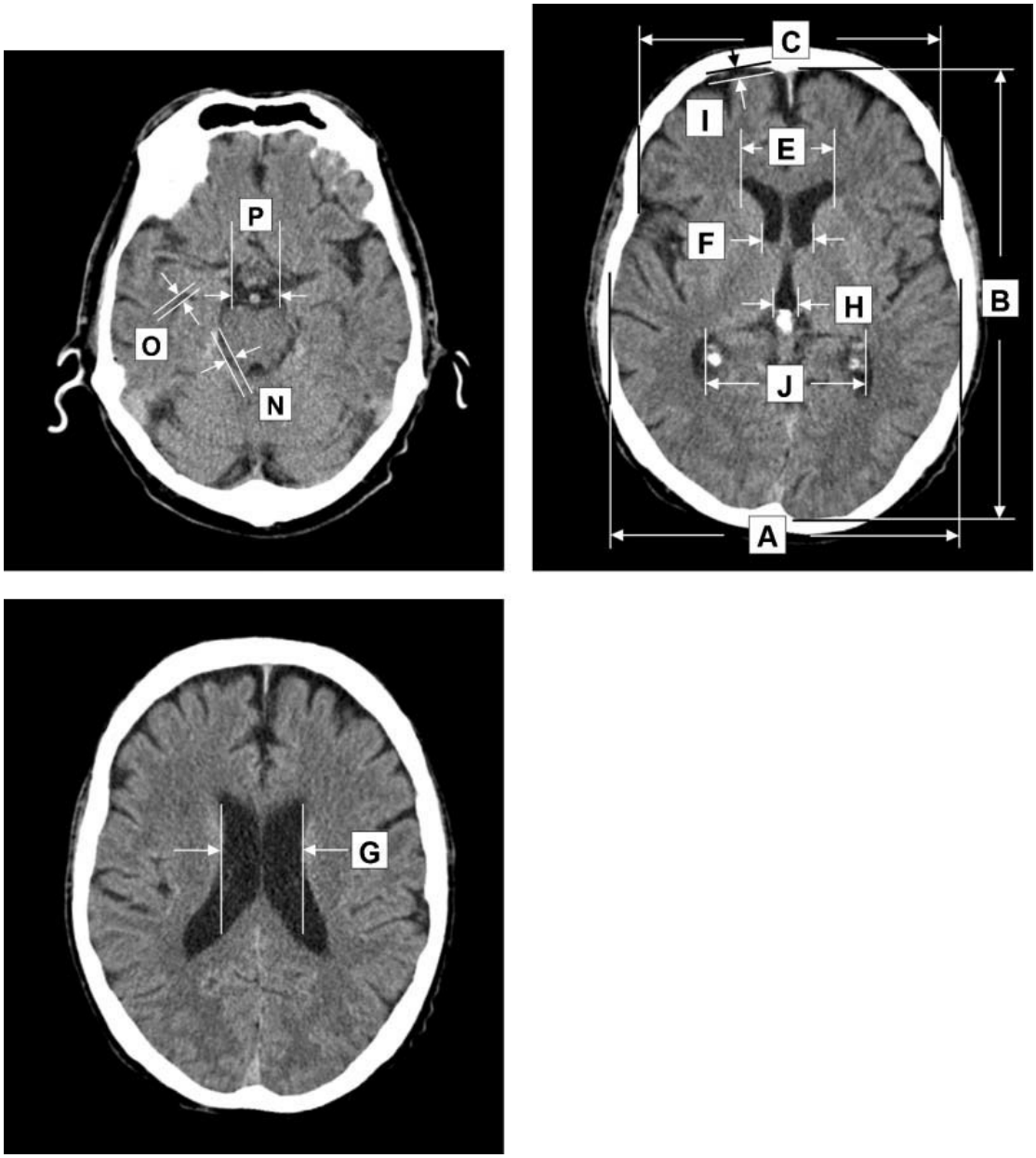


Figure 9.1 Linear measurements taken on axial CT slices Reproduced with permission from Acta radiologica (SAGE publications –see appendix 9 for permission information)

A- Maximal transverse intracranial width
B- Maximal longitudinal intracranial width
C- Maximal width of frontal skull
H- Maximal width of the third ventricle

I- Maximal width of frontal subarachnoid space
P- Temporal horn diameter

O- Suprasellar cistern width
N- Width of cistern ambiens

E- Maximal frontal horn width
F- Minimal intercaudate distance
J- Distance between the choroid plexuses
G- Minimal ventricular body width
M- Sum of widths of four widest sulci (image not shown)

9.3.2 Reliability

Intrarater reliability was tested by comparing repeated measurements from 10 subjects (subjects 1, 10, 20, 30, 40, 50, 60, 70, 80 and 90 were selected). The interval between the measurements was 4 weeks.

Interrater reliability was tested by comparing repeated measurements for 20 subjects (including the 10 used for the intrarater reliability test) using a second rater (Dr Karen Ferguson, Department of Geriatric Medicine, University of Edinburgh, UK), blind to the first measurements.

9.3.3 Statistical analysis

The relationship between linear measurements of atrophy and atrophy ratings (none, mild, moderate or severe, as previously described) was investigated using Spearman's correlations (one-tailed). The intrarater and interrater reliability was investigated using Spearman's correlations and intraclass correlation coefficients (ICC, using a fixed effects two way mixed model, with absolute agreement). The ICC was interpreted as follows: >0.75=excellent agreement, 0.4-0.75=fair to good agreement, <0.4= poor agreement (Altman, 1991). Despite some measurements showing poor agreement between raters, all were included in the initial analysis of relationships between linear measures and atrophy rating scores and between linear measures and delirium, because this study is exploratory in nature.

The relationship between the linear CT measurements and delirium at any time point in the 12 months after stroke (binary outcome, yes or no) was initially investigated using point biserial correlations. Principle Component Analysis (PCA) was then considered for analysis of the data, because of potential collinearity when considering a multivariate regression analysis. However, the data were not suitable for PCA because of the sample size (n=95, the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy=0.41, KMO should be >0.5 to proceed (Field, 2009)). Thus the assumptions required for multivariate logistic regression were tested. The assumption of linearity of the logit was met, with all continuous variables being linearly related to the log of the outcome variable (delirium). Multicollinearity was then tested for;

tolerance values were all greater than 0.1 and variance inflation factor values were all below 10 and the variance proportions do not cluster to one eigenvalue, all of which indicates that collinearity is not a concern (Field, 2009). Multivariate analysis was then performed using binary logistic regression using the enter method, with age, sex, NIHSS, IQCODE (variables selected a priori) and the linear measurements which showed a significant correlation with delirium (taken to be a p value of <0.01) in the univariate analysis being included in the model. This approach was taken, rather than using specific linear measurements selected a priori, because there is no previous data to guide an a priori approach, and so the analysis should be considered exploratory.

9.4 Results

The brain scans for 95 participants were included in the study. Baseline characteristics of the participants are outlined in chapter 5. The linear measurements are outlined in table 9.1. The values for median measurements for all parameters are similar to those reported by Zhang and colleagues (Zhang, 2008).

Table 9.1 Linear measurements taken from baseline CT brain scans

Linear measurement	Alphabetical annotation	Median (mm)	IQR (mm)
Maximal transverse intracranial width	A	138.3	134.1-141.6
Maximal longitudinal intracranial width	B	157.1	151.4-161.6
Maximal width of frontal skull	C	106.2	101.5-111.9
Maximal frontal horn width	E	38.6	35.2-41.5
Minimal intercaudate distance	F	19.4	16.9-23.7
Minimal ventricular body width	G	27.0	23.8-31.9
Maximal width of the third ventricle	H	8.1	7.2-10.0
Maximal width of frontal subarachnoid space	I	4.0	3.5-5.1
Distance between the choroid plexuses	J	53.6	47.7-58.9
Sum of the widths of the four widest sulci	M	20.2	17.8-23.3
Width of the cistern ambiens	N	4.3	3.9-5.2
Temporal horn diameter	O	4.4	3.7-5.4
Supracellar cistern width	P	25.8	24.2-28.1
Evans ratio	E/C	0.4	0.3-0.4
Bicaudate ratio	F/A	0.14	0.12-0.17
Huckman number	E+F	59.3	52.9-64.9
Cella media index	G/A	0.2	0.17-0.23
Third ventricular ratio	H/A	0.06	0.05-0.07
Ventricle index	J/E	1.4	1.2-1.5
Frontal subarachnoid ratio	I/B	0.03	0.02-0.03
Four cortical sulci ratio	M/A	0.15	0.13-0.17
Cistern ambiens ratio	N/A	0.03	0.03-0.04
Temporal horn ratio	O/A	0.03	0.03-0.04
Supracellar cistern ratio	P/A	0.19	0.17-0.20

9.4.1 Intra-rater reliability

As can be seen from table 9.2, intrarater agreement was excellent for the majority of measurements with the exception of the maximal width of frontal skull, C (fair to good agreement), width of the cistern ambiens, N (fair to good agreement) and the temporal horn diameter, O (poor agreement).

9.4.2 Inter-rater reliability

As can be seen from table 9.3, interrater reliability was excellent for maximal skull width and frontal skull width, minimal intercaudate distance, maximal width of the third ventricle and the sum of the widths of the four widest sulci. There was fair to good agreement for the maximal frontal horn width, minimal ventricular body width, minimal width of the frontal subarachnoid space and the temporal horn diameter, and the remainder of the measurements showed poor interrater reliability.

9.4.3 Correlations between linear measurements and global atrophy rating score

All of the linear measurements showed a statistically significant correlation with the atrophy rating score (table 9.4), with the exception of measurements A, B, C, J and P. Correspondingly, all derived indices and ratios showed a significant correlation with the atrophy rating score, with the exception of I/B and P/A.

9.4.4 Correlations between linear measurements and diagnosis of delirium at any point during the study (univariate analysis)

As it was specified a priori that a p value of ≤ 0.01 would be considered significant, only the maximal longitudinal intracranial width and the cisterns ambiens ratio correlated significantly with a diagnosis of delirium at any point during the study (table 9.5).

Table 9.2 Intraclass correlation (ICC) and Spearman's correlations for intrarater reliability

Linear Measurement	Alphabetical annotation	Spearman's rho	p	ICC
Maximal transverse intracranial width	A	0.823	0.003	0.931
Maximal longitudinal intracranial width	B	0.927	<0.001	0.915
Maximal width of frontal skull	C	0.539	0.108	0.685
Maximal frontal horn width	E	0.903	<0.001	0.881
Minimal intercaudate distance	F	0.673	0.033	0.861
Minimal ventricular body width	G	0.770	0.009	0.953
Maximal width of the third ventricle	H	0.600	0.067	0.882
Maximal width of the frontal subarachnoid space	I	0.636	0.048	0.755
Distance between the choroid plexuses	J	0.915	<0.001	0.780
Sum of the widths of the four widest sulci	M	0.661	0.038	0.828
Width of cistern ambiens	N	0.467	0.174	0.660
Temporal horn diameter	O	0.273	0.446	0.227
Supracellar cistern width	P	0.758	0.011	0.771

[+]Poor agreement
[++]Fair to good agreement
[+++]Excellent agreement

Table 9.3 Intraclass correlation (ICC) and Spearman's correlations for interrater reliability

Linear Measurement	Alphabetical annotation	Spearman's rho	p	ICC
Maximal transverse intracranial width (A)	A	0.715	<0.001	0.660
Maximal longitudinal intracranial width (B)	B	0.655	0.002	0.376
Maximal width of frontal skull (C)	C	0.740	<0.001	0.859
Maximal frontal horn width (E)	E	0.570	0.009	0.414
Minimal intercaudate distance (F)	F	0.802	<0.001	0.858
Minimal ventricular body width (G)	G	0.486	0.03	0.653
Maximal width of the third ventricle (H)	H	0.703	0.001	0.847
Maximal width of the frontal subarachnoid space (I)	I	0.507	0.023	0.661
Distance between the choroid plexuses (J)	J	0.247	0.295	0.039^{+}
Sum of the widths of the four widest sulci (M)	M	0.678	0.001	0.813
Width of cistern ambiens (N)	N	0.038	0.872	0.142
Temporal horn diameter (O)	O	0.610	0.004	0.579
Supracellar cistern width (P)	P	0.180	0.446	0.082

Table 9.4 Spearman's rho correlations between linear measurements and global atrophy rating scores (none, mild, moderate or severe)

Linear measurement	Alphabetical annotation	Spearman's rho	p
Maximal transverse intracranial width	A	0.116	0.13
Maximal longitudinal intracranial width	B	0.130	0.105
Maximal width of frontal skull	C	0.130	0.104
Maximal frontal horn width	E	0.563	<0.001
Minimal intercaudate distance	F	0.600	<0.001
Minimal ventricular body width	G	0.522	<0.001
Maximal width of the third ventricle	H	0.530	<0.001
Maximal width of frontal subarachnoid space	I	0.194	0.030
Distance between the choroid plexuses	J	0.097	0.176
Sum of the widths of the four widest sulci	M	0.192	0.031
Width of the cistern ambiens	N	0.212	0.020
Temporal horn diameter	O	0.416	<0.001
Supracellar cistern width	P	0.084	0.208
Evans ratio	E/C	0.504	<0.001
Bicaudate ratio	F/A	0.606	<0.001
Huckman number	E+F	0.609	<0.001
Cella media index	G/A	0.518	<0.001
Third ventricular ratio	H/A	0.528	<0.001
Ventricle index	J/E	-0.390	<0.001
Frontal subarachnoid ratio	I/B	0.144	0.083
Four cortical sulci ratio	M/A	0.173	0.047
Cistern ambiens ratio	N/A	0.191	0.032
Temporal horn ratio	O/A	0.377	<0.001
Supracellar cistern ratio	P/A	0.072	0.245

Table 9.5 Univariate analysis of the relationship between linear measurements on CT brain scans and diagnosis of delirium at any point after stroke (binary outcome, yes or no) n=95.

Linear measurement	Alphabetical annotation	r_{pb}	p
Maximal transverse intracranial width	A	-0.157	0.128
Maximal longitudinal intracranial width	B	-0.300	0.003
Maximal width of frontal skull	C	-0.131	0.206
Maximal frontal horn width	E	0.132	0.204
Minimal intercaudate distance	F	0.113	0.278
Minimal ventricular body width	G	-0.017	0.872
Maximal width of the third ventricle	H	0.197	0.057
Maximal width of frontal subarachnoid space	I	0.229	0.026
Distance between the choroid plexuses	J	-0.113	0.277
Sum of the widths of the four widest sulci	M	0.101	0.328
Width of the cistern ambiens	N	0.208	0.043
Temporal horn diameter	O	0.181	0.080
Supracellar cistern width	P	-0.017	0.869
Evans ratio	E/C	0.214	0.038
Bicaudate ratio	F/A	0.146	0.159
Huckman number	E+F	0.031	0.765
Cella media index	G/A	-0.016	0.877
Third ventricular ratio	H/A	0.203	0.049
Ventricle index	J/E	-0.228	0.027
Frontal subarachnoid ratio	I/B	0.240	0.020
Four cortical sulci ratio	M/A	0.135	0.193
Cistern ambiens ratio	N/A	0.323	0.001
Temporal horn ratio	O/A	0.190	0.067
Supracellar cistern ratio	P/A	0.013	0.898

r_{pb}= point-biserial correlation coefficient

9.4.5 Multivariate analysis

A logistic regression analysis was performed (table 9.6) with delirium as the dependant variable and age, sex, NIHSS, IQCODE (positive or negative) and the linear measurements which showed a significant correlation with delirium in the univariate analysis (with a p value of <0.01) as predictor variables. A total of 95 cases were analysed (omnibus chi-square = 40.63, df = 6, $p < 0.0005$). The model accounted for between 35.1% and 51.2% of the variance in the delirium status. Table 9.6 gives the Odds Ratios, 95% confidence internals and associated probability values for each of the predictor variables. This shows that only NIHSS score and the cistern ambiens ratio (N/A) were associated with delirium.

Table 9.6 Multivariate analysis: Binary logistic regression model. Predictors of delirium at any time point after stroke (n=95)

Predictor variable	Odds Ratio	CI	p
Maximal longitudinal intracranial width (B)	0.90	0.80-1.00	0.190
Cistern ambiens ratio (N/A)	1.41	1.48-4.96	0.028
Age (years)	1.09	1.00-1.19	0.075
Sex (being female)	0.65	0.14-3.13	0.536
IQCODE (positive or negative)	1.83	0.13-25.74	0.653
NIHSS score (per point)	1.23	1.06-1.43	0.006

9.5 Discussion

In this study, linear measurements in general showed excellent correlation with atrophy rating scores, with the exception of those measurements which are fixed and so would not alter in an atrophied brain (for example, skull dimensions, measurements A,B and C). In multivariate analysis, when age, sex, IQCODE and NIHSS scores are controlled for, only the cistern ambiens ratio was associated with delirium. NIHSS score (as a measure of stroke severity) also significantly predicted delirium when all other factors were controlled for.

Intrarater reliability was good or excellent for all linear measurements, with the exception of the temporal horn diameter, which showed poor agreement. This may be due to the small overall size of the temporal horn, meaning that any small differences in measurements causes a large percentage difference between measurements. Furthermore, small changes in the level at which the CT slice is being measured would also have a large effect on the measurement. In future studies, it would be prudent to exclude those measures which showed poor reliability from the methodology.

Interrater reliability was good or excellent for nine out of thirteen measurements. The measurements that showed poor interrater reliability were; maximal longitudinal intracranial width (B), distance between the choroid plexuses (J), width of cistern ambiens (N) and supracellar cistern width (P). These measurements all have the potential to vary quite significantly depending upon the slice selected to measure and it is possible that this, at least in part, accounts for the poor reliability. Furthermore the distance between the choroid plexuses relies on a subjective judgement of where the outer edge of the plexus begins, and this can be particularly difficult to judge if there is no calcification to demarcate the edges.

Both intrarater reliability and interrater reliability can be calculated using statistical methods. The most appropriate test to use depends largely on the type of assessment being compared and whether raters are selected randomly from a population of potential raters or are fixed raters. For categorical variables, whereby the aim is to compare the ability of the raters to classify subjects into one of several groups, the measure of agreement most frequently used is kappa. Kappa is calculated by taking

the agreement that would be expected by chance and expressing the actual agreement found in the study as a proportion of the possible scope for doing better than chance. Thus when agreement is perfect, the kappa value will be 1 and when there is no agreement, the value is 0. As a guideline, Altman (Altman, 1991) suggests that a value of between 0.21-0.40 is fair agreement, 0.41-0.6 is moderate agreement, 0.61-0.8 is good agreement and 0.81-1.00 is excellent agreement. Weighted kappa statistics are useful when the degree of disagreement between raters is important. For example if looking at the brain atrophy rating scores presented in chapter 8 it is helpful to take account of whether raters disagreed by one category (for example one rated atrophy as mild and one as moderate) or by more than one category (one rated the scan as mild atrophy and one as severe, for example). In order to calculate weighted kappa scores the degree of difference is given a weighting score. The kappa statistic cannot be used if the outcome variable is continuous, as the raters are not classifying subjects into groups. For continuous variables (such as the linear measurements investigated in this study), intraclass correlations coefficients (ICC) are a suitable alternative (Shrout and Fleiss, 1979). Intraclass correlations coefficients represent the proportion of variance in a set of scores that is attributable to the true score variance. The ICC is calculated by using ANOVA to test whether the 2 sets of scores are significantly different to each other, and the ANOVA results are then put into a fixed effects model to calculate the ICC. Thus an ICC of 0.95 implies that 5% of the variance seen in the observed scores is due to observer error, whereas a score of 0.10 would imply that 90 % of the variance seen between observed scores is due to observer error. Bland- Altman plots are a third statistical method often used to compare measurements in studies. Bland-Altman plots are used to compare two different methods of measurement, for example they could be used to compare salivary cortisol levels measured by two different sets of laboratory equipment. They can also be used to analyse the repeatability of a single measurement and to compare measurements between two observers, although they were not developed to be used in this way (Bland and Altman, 1986).

The finding that the cistern ambiens ratio is the only measurement that predicts delirium is intriguing. However, it must be noted that the cistern ambiens ratio showed poor interrater agreement, and also that in multivariate analysis, the 95%

confidence intervals for this measurement were wide, and there was only a small change in cistern ambiens size per unit change in delirium status. Thus this finding should not be over-interpreted, particularly in the context of this small exploratory study. Zhang and colleagues (Zhang, 2008), who first described the methodology used here for linear measurements, looked at those with Alzheimer's disease (AD) compared with controls and found that those with AD had a greater temporal horn ratio and supracellar cistern ratio when compared to controls. This finding is in keeping with medial temporal lobe atrophy, one of the known pathological changes found in AD. The ambient cistern is part of the subarachnoid cisterns, and widening of the cistern results from atrophy of the structures which border it (Chakeres, 1986), in particular the cerebellum, midbrain and pons. Furthermore, the cistern ambiens laterally extends to become the transverse fissure, which is one of the perihippocampal fissures (Chakeres, 1986). Atrophy of the perihippocampal fissures has been shown to be associated with ageing and AD (Li, 2006). Interestingly, neither the cistern ambiens measurement nor the cistern ambiens ratio correlated significantly with the atrophy rating scores in univariate analysis. This may be explained, at least in part, by the fact that although the rater takes account of the whole brain when rating for atrophy, atrophy of the cistern ambiens is likely to be much more subtle than enlarged ventricles, for example. This would mean that the cistern ambiens rating would contribute little to the overall rating given to the brain scan.

The maximal longitudinal intracranial width was also associated with delirium in univariate analysis, although this relationship did not hold true when other factors such as age and stroke severity were controlled for in the multivariate analysis. Furthermore, it is not the absolute measurement of the cistern ambiens that was associated with delirium in this study, but rather the ratio of the cistern to the maximum transverse intracranial width. This means that both measurements that are associated with delirium in the univariate analysis include a measure of head size. Head size is fixed throughout adulthood, whilst brain size tends to decrease due to age-related atrophy and intracranial area (ICA) varies considerably between individuals and is an estimate of peak or maximal brain volume (Shenkin, 2009), which is attained at around 6 years of age (Gale CR, 2003). This is important as it has previously been demonstrated that whole brain volume correlates positively with current intelligence (Shenkin, 2009) and also that ICA correlates positively with

general cognitive ability (MacLullich, 2002). This means that the associations found with delirium here may simply reflect a participant's cognitive reserve, as a function of their general cognitive ability.

It is perhaps surprising that other linear measurements do not seem to be associated with delirium, considering that the overall atrophy rating was. One may perhaps have expected measurements such as the ventricular body width to show an association with delirium. It may simply be that the absolute differences in measurements between those with and without delirium are subtle, and so no clear association emerges, whereas with the atrophy rating scale these subtleties are accounted for by the rater. Finally, and perhaps most importantly, it should be noted that this is a small pilot study, with only those who have recently had a stroke included, meaning that the findings should be interpreted with caution. Larger studies which are appropriately powered to investigate the associations between linear measures on CT and delirium will be required to unpick these relationships, and this study should be considered to be exploratory.

Chapter 10: General Discussion

The studies presented in this thesis aimed to investigate: 1).The relationship between delirium after stroke and cortisol, 2). The relationship between delirium, cortisol and cognitive changes in the year after stroke, and 3). Baseline changes on brain CT scans and their relationship to delirium. The studies specifically aimed to investigate whether delirium after stroke is associated with elevated cortisol levels, the trajectory of cognitive function after stroke, and the relationship between cognition, delirium and cortisol levels. Finally, the associations between baseline features seen on admission CT brain scans (specifically white matter lesions, brain atrophy and the presence of an acute and/or chronic stroke lesion) and development of delirium were investigated, and an objective method of assessing brain atrophy on CT brain scans after stroke was piloted.

This concluding chapter will summarise the main findings of this thesis, will discuss the studies strengths and limitations, and will describe how the findings contribute to existing knowledge.

10.1 Main findings

As was expected, following my systematic review of the previous literature (chapter 2), there was an elevation in cortisol levels in the first 7 days after stroke, in a proportion of participants. This was more marked in the participants who developed delirium, however when other confounders were controlled for, such as age and stroke severity, cortisol was not found to be independently associated with delirium. There was also a trend towards loss of diurnal variation in cortisol levels in the delirium group, but again this was not statistically significant once other confounders had been controlled for. The overall cognitive trajectory for all participants showed a degree of improvement over the 12 month follow-up period. Those in the delirium group tended to have poorer cognitive function at the outset, and although there was a trend towards improvement in cognition, particularly for those who had a mild or moderate severity delirium, this is confounded by attrition of those with more severe cognitive impairment. Delirium was associated with a lower MoCA score overall, however

incident delirium did not have a significant effect on the trajectory of the MoCA over the 12 month follow-up period. An association was found between baseline global cognition after stroke (measured by the MoCA score at the time of recruitment) and the loss of the normal diurnal pattern of cortisol levels. Brain atrophy, seen on admission CT brain scans, was associated with the development of delirium after stroke, however other changes, such as white matter lesions and the presence of a visible acute stroke lesion, were not. Finally, linear measurements of brain regions, as an objective measurement of brain atrophy, had good inter-rater reliability, but correlated only fairly with qualitative assessments of brain atrophy, and linear measurements were not associated with delirium in multivariate analysis.

The studies presented in this thesis are all observational cohort studies. This means that it is not possible to infer the direction of causation for the findings. For example, it may be the case that those with brain atrophy were predisposed to developing delirium after stroke because of undiagnosed (and perhaps subtle) cognitive decline, which was not picked up by the IQCODE, rather than brain atrophy per se being the causative factor. Confounders were controlled for in multivariate analysis where possible, however the number of confounders that it is possible to control for is limited by the number of participants in the study (Field, 2009), and is also limited to factors that it is possible to quantify. Although I was not able to demonstrate a statistically significant relationship between cortisol and delirium in this thesis, it remains possible that cortisol is amongst the underlying mechanisms that lead to delirium, but that the effect size is smaller than the 'moderate effect' that the study was powered for. It may be that other factors that I did not measure in these studies, such as inflammation, play an important role in the pathogenesis of delirium (Maldonado, 2013), and that this may occur alongside any effects of HPA axis dysregulation.

Perhaps the most unexpected finding in this thesis has been that the global cognitive trajectory for the whole cohort improved over the course of the 12 months. The magnitude of the improvement for the whole cohort, after controlling for the presence of delirium, was small and probably not clinically significant (an increase of 1.09 points on the MoCA scale across the year), however, when participants who developed delirium were stratified by delirium severity, some groups showed a clear,

clinically significant, improvement. For example, for those who developed a mild delirium (n=8), the median MoCA score at baseline was 18, and at 12 months the median score for this group (n=7) was 25.5. There was a trend towards improvement in the group of participants who never developed delirium (median baseline MoCA 24, Median MoCA at 12 months 26), although again this is probably not clinically significant. As no pre-morbid cognitive testing was available, it can only be said that any improvement occurred from the time of recruitment, and it was not possible to say whether there had been any overall improvement or deterioration at 12 months compared to pre-stroke cognitive function. However, for those who survived to 12 months, this may have important implications for follow-up and discharge planning. It must be noted that these results should be treated with extreme caution, as attrition of those with the poorest cognitive function has skewed the results at follow-up (this has been termed "survival bias." (Weuve et al., 2015)), and there may also be a learning effect for the cognitive tests, resulting in artificially higher results at 12 months. Furthermore, although depression was not specifically tested for in the studies, it is known that depression is common after stroke, affecting around 30% in the year after stroke, falling to around 25% between 1 and 5 years post-stroke, meaning that some of the improvement may have been explained by resolving depression (Hackett and Pickles, 2014). Even accounting for these factors, a proportion of participants undoubtedly showed improvement in global cognitive function over the 12 month follow-up period. Resolution of the acute effects of a stroke and of delirium probably accounts for much of the improvement seen and it is interesting to note that the improvement continued between 1 month and 4 months, and to a lesser extent, between 4 months and 12 months, which was not hypothesised, based on previous literature. As previously discussed much of the previous literature investigating cognition after stroke has not accounted for the effect of delirium on cognitive function. This is important as I have found that each episode of delirium in this study was associated with a 5 point decrease in the MoCA score overall, although delirium did not have an effect on the rate of change (or trajectory) of the MoCA over the 12 months.

10.2 Strengths and limitations

10.2.1 Strengths

The studies presented in this thesis have several strengths. A service user group helped to ensure that the study was acceptable to participants, and their valuable insights also helped to retain participants for the duration of the study (89 % completed the study in total). This relatively high follow-up rate was achieved largely because I made home visits to participants, ensuring that the study was as acceptable as possible to a frail cohort, many of whom were left with a disability after stroke.

The methods used for assessing delirium were frequent, detailed, and attempted to determine the presence, duration and severity of delirium in a methodical way, incorporating both subjective and objective methods of assessment, as well as detailed assessment of level of alertness. This very detailed and structured method of assessment has not been used in studies of delirium after stroke before, although a similar methodology has been used in a study of delirium after hip fracture (Hall et al., 2015). Furthermore, delirium assessments were also performed at follow-up visits and, where possible, episodes of delirium between assessment points at 4 months and 12 months were ascertained from informants, clinical notes and General Practitioners. Again, this detailed method which attempts to capture the natural history of delirium after stroke, has not previously been attempted, although again a similar methodology has been employed in a study of delirium after hip fracture (Neerland et al., 2016).

In contrast with previous studies of delirium after stroke, patients who had a reduced level of consciousness were included in the study. This is essential, as drowsiness is commonly seen in those with delirium (Chester et al., 2012). Care was taken to ensure that delirium was only diagnosed strictly by DSM-IV criteria, if there was a change in the patient's condition, from what may be thought of as their new baseline after stroke. In this way it was possible to include those with severe TACS, and the results are therefore more generalizable to the acute stroke unit population. Delirium after stroke is particularly challenging to study in those with neurological deficits such as aphasia and inattention, as the core criteria for diagnosis of delirium rely on assessment of organized thinking and of attention (McManus et al., 2007). However, using the very careful detailed assessments described in this thesis, it was possible to apply the DSM-IV criteria to all participants, although none of those included had a very dense aphasia. The study protocol was planned such that those with a very dense

aphasia would be excluded from the study if there was no evidence of improvement over the first 2 weeks after stroke, but in practice it was not necessary to exclude any of those recruited for this reason.

10.2.2 Limitations

There are some important limitations to the studies described in this thesis. One of the major limitations is the numbers of participants recruited. Extreme care was taken in the planning and execution of the study, including the formation of a service user group, to try and maximise recruitment given the constraints of a single centre study and a relatively frail target population. Every effort was made to recruit all eligible participants, but inevitably, this meant approaching frail, elderly and extremely unwell people, many of whom did not have the capacity to provide informed consent. As outlined in previous chapters, this made timeous recruitment challenging, and also led to large numbers of proxies being approached to provide consent (with large numbers of proxies going on to refuse consent). Proxies may have found it particularly difficult to give consent for a study which had a 1 year follow-up period, which may have seemed onerous, and may also have been reluctant to provide consent for a study which was not providing obvious potential benefit for participants, in the way that inclusion to a randomised controlled trial may be perceived to have potential benefits. The potential benefits of careful follow-up of cognitive function, as well as the benefits of early detection of delirium in the acute phase, were fully explained, however many proxies felt that their relative was too unwell to take part in any study requiring multiple follow-up visits. These challenges, many of which apply to all longitudinal studies of delirium, are difficult to overcome, although integrating delirium assessment into routine clinical care may be one method of reducing the perceived burden of studies. The small study size also meant that subgroup analysis was difficult, with some groups having only a few members by the 12 month follow-up. This means that some of the sub-studies must be seen as exploratory. Recruitment and follow-up in the acute phase after stroke was all done by a single assessor. This has some benefits, in that there is likely to be good internal consistency for delirium assessments, however it did mean that the number of assessments had to be limited. It is therefore possible that a brief episode of delirium could have been overlooked,

although casenote analysis and informant and staff interviews were performed in a semi-structured way in order to minimise omission.

10.3 Contribution to existing knowledge

This thesis presents the largest study to date to investigate the associations between cortisol after stroke and delirium. Furthermore, it provides one of the most detailed assessments for delirium after stroke to have been used in the literature, and also includes the longest follow-up period of any of the studies of cortisol and delirium after stroke. The study of CT brain scans has provided additional and complementary detail to the previous studies which have found an association between brain atrophy and delirium after stroke (Oldenbeuving, 2011, Henon et al., 1999). Finally it has added to our knowledge of the trajectory of global cognition after stroke, and in particular has highlighted the importance of assessing for delirium when testing cognition after stroke, and also the importance of re-assessment- particularly if decisions about discharge placement have been based on cognitive function

It seems unlikely to me that any single pathophysiological factor is entirely responsible for the development of delirium. Whilst cortisol may well have a role to play, other factors such as the underlying vulnerability of the brain, direct insults to the brain, and peripheral (and central) inflammation all probably play their part to a greater or lesser extent (Maldonado, 2013). This complexity means that it is extremely challenging to establish which of these factors is the most important in any patient population. Furthermore, those most at risk of delirium tend to either be elderly, with multiple co-morbidities, or be extremely unwell, perhaps requiring Intensive Care treatment. This adds another layer of complexity as illness, polypharmacy and environmental factors all may play a part in the development and maintenance of delirium. Unravelling which few factors are the most important and, crucially, determining which of these are safely modifiable is the challenge now facing the field of delirium research. Potential directions for future work will be discussed in detail in the following section (section 10.4)

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10.4 Future work

There is a clear need to further unpick the underlying pathophysiology of delirium after stroke, as the impact of delirium on outcomes after stroke is so devastating for patients, and understanding the pathophysiology offers potential for therapeutic targets. The evidence presented in this thesis does not provide a strong enough case for trials of treatments aimed solely at lowering cortisol levels, or dampening the HPA axis response, to be considered. A larger prospective study to investigate the role of cortisol in delirium after stroke (perhaps in combination with other factors such as markers of inflammation) may be fruitful, given the trend towards higher cortisol in the delirium group in this study, but this would require a much larger population based study, allowing participants to be recruited prior to stroke, to have cognitive testing and cortisol levels measured, and then to follow those that do have a stroke into the hospital setting (if we set α at 0.05 a sample size of 220 would be required to provide 80% power to detect a difference in cortisol levels between the two groups, and a sample size of 300 would be needed to provide 90% power). This would be difficult and costly to do, as a large initial sample would be required, in order that the study have sufficient power to detect any differences in cortisol between those with and those without delirium. However, the benefits of identifying key underlying pathophysiological processes are great, offering the potential to reduce not only the burden of delirium incidence for stroke patients, but also potentially reducing the burden of cognitive decline after stroke, which is a key priority for both stroke survivors and their carers (Pollock et al., 2014).

Despite the lack of clarity surrounding the pathophysiology of delirium after stroke, given its high prevalence and association with adverse outcomes, it may be more fruitful to pursue a pragmatic multi-component intervention study, similar to that used in the Hospital Elder Life Program (Inouye et al., 2000), in the stroke unit. Some aspects of this may be challenging in stroke patients, for example nutritional needs are often being met by nasogastric feeding, however many of its components would be feasible. I personally feel that these types of intervention studies may in fact prove more useful, with tangible and timely benefits to patients, than studies attempting to untangle the complex underlying pathophysiology of delirium.

The neuroimaging studies presented in this thesis, alongside other similar work, provide intriguing possibilities in terms of determining the brain structural risk factors for delirium in more detail. A deeper understanding of whether there are specific pathological changes which predispose individuals to developing delirium (and indeed dementia) may allow these factors to be targeted as preventative measures. The neuroimaging studies presented in this thesis have demonstrated the feasibility of obtaining CT brain images for the whole of the target population (there were no participants who were unable to have a CT brain scan performed) and also the feasibility of subsequent clinical correlation to assess for evidence of delirium. Computed Tomography remains an attractive modality for investigating neuroimaging changes in delirium because of its acceptability to patients, even those who are acutely unwell. As CT technology improves, scans are becoming more fine grained in the detail they can provide. It may soon be possible, for example, to delineate the hippocampus on CT images, in much the same way that it is possible to do on MRI scans currently. This presents exciting opportunities in the field of delirium research, particularly after stroke, as it offers the possibility of scanning those who are very unwell (and thus unable to tolerate MRI) and to map out any acute structural changes that may take place during an episode of delirium, in crucial regions such as the hippocampus. This real-time scanning during delirium has not been attempted in stroke patients, and although challenging, has the potential to dramatically improve our understanding of the structural brain changes which take place during an episode of delirium. Ideally one would also have pre and post delirium scans for comparison, and it is therefore probable that this type of study would require a large population based cohort.

In conclusion, the findings from this thesis are relevant to important areas of delirium research, including the diagnosis of delirium after stroke, the pathophysiological mechanisms of delirium after stroke and potential avenues for further investigation of the predisposing factors which lead to the development of delirium. I would now like to utilise the knowledge and skills gained from these studies to undertake a randomized controlled trial of a multicomponent intervention for delirium prevention after stroke. The study would require detailed delirium assessments for all participants and, as all participants would have had a CT brain scan, it would also be possible to

include detailed baseline CT analysis, and possibly follow-up CT brain scanning. The multicomponent intervention would need to be tailored to the stroke population, but would be based on those successfully used in studies based in general medical wards such as that outlined in the Hospital Elder Life Program (Inouye et al., 2000) . The primary outcome from the study would be delirium incidence, with secondary outcomes including delirium severity and morbidity and mortality.

11. References

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Appendix 1: Search strategy for systematic literature review of glucocorticoids and stroke (chapter 2)

Part A: Stroke search strings (Cochrane Stroke Group)

1. cerebrovascular disorders/
2. exp basal ganglia cerebrovascular disease/
3. exp brain ischemia/
4. exp carotid artery diseases/
5. cerebrovascular accident/
6. exp brain infarction/
7. exp cerebrovascular trauma/
8. exp hypoxia-ischemia, brain/
9. exp intracranial arterial diseases/
10. intracranial arteriovenous malformations/
11. exp "Intracranial Embolism and Thrombosis"/
12. exp intracranial hemorrhages/
13. vasospasm, intracranial/
14. vertebral artery dissection/
15. aneurysm, ruptured/
16. brain injuries/
17. brain injury, chronic/
18. exp carotid arteries/
19. endarterectomy, carotid/ or endarterectomy/
20. *heart septal defects, atrial/
21. *atrial fibrillation/
22. (stroke or poststroke or post-stroke or cerebrovasc$ or brain vasc$ or cerebral vasc$ or cva$ or apoplex$ or isch?emi$ attack$ or tia$1 or neurologic$ deficit$ or SAH or AVM).tw.
23. ((brain$ or cerebr$ or cerebell$ or cortical or vertebrobasilar or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or MCA or anterior circulation or posterior circulation or basal ganglia) adj10 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypox$ or vasospasm or obstruction or vasculopathy)).tw.
24. ((lacunar or cortical) adj5 infarct$).tw.
25. ((brain$ or cerebr$ or cerebell$ or intracerebral or intracran$ or parenchymal or intraventricular or infratentorial or supratentorial or basal gangli$ or subarachnoid or putaminal or putamen or posterior fossa) adj10 (haemorrhage$ or hemorrhage$ or haematoma$ or hematoma$ or bleed$)).tw.
26. ((brain or cerebral or intracranial or communicating or giant or basilar or vertebral artery or berry or saccular or ruptured) adj10 aneurysm$).tw.
27. (vertebral artery dissection or cerebral art$ disease$).tw.
28. ((brain or intracranial or basal ganglia or lenticulostriate) adj10 (vascular adj5 (disease$ or disorder or accident or injur$ or trauma$ or insult or event))).tw.

29. ((isch?emic or apoplectic) adj5 (event or events or insult or attack$)).tw.
30. ((cerebral vein or cerebral venous or sinus or sagittal) adj5 thrombo$).tw.
31. (CVDST or CVT).tw.
32. ((intracranial or cerebral art$ or basilar art$ or vertebral art$ or vertebrobasilar or vertebral basilar) adj5 (stenosis or isch?emia or
insufficiency or arteriosclero$ or atherosclero$ or occlus$)).tw.
33. ((venous or arteriovenous or brain vasc$) adj5 malformation$).tw.
34. ((brain or cerebral) adj5 (angioma$ or hemangioma$ or haemangioma$)).tw.
35. carotid$.tw.
36. (patent foramen ovale or PFO).tw.
37. ((atrial or atrium or auricular) adj fibrillation).tw.
38. asymptomatic cervical bruit.tw.
39. exp aphasia/ or anomia/ or hemiplegia/ or hemianopsia/ or exp paresis/ or deglutition disorders/ or dysarthria/ or pseudobulbar
palsy/ or muscle spasticity/
40. (aphasi$ or apraxi$ or dysphasi$ or dysphagi$ or deglutition disorder$ or swallow$ disorder$ or dysarthri$ or hemipleg$ or hemipar$
or paresis or paretic or hemianop$ or hemineglect or spasticity or anomi$ or dysnomi$ or acquired brain injur$ or hemiball$).tw.
41. ((unilateral or visual or hemispatial or attentional or spatial) adj10 neglect).tw.
42. or/1-41

Part B: Cortisol search strings
43. Hydrocortisone/
44. cortisol.tw.
45. s-cortisol.tw.
46. s?cortisol.tw.
47. serum-cortisol.tw.
48. cortisone.tw.
49. corticosteroid.tw.
50. glucocorticoid*.tw.
51. epicortisol.tw.
52. stress response.tw.
53. hypercortisol?emia.tw.
54. or/43-53

Appendix 2: Results of the STROBE quality assessment for articles included in systematic literature review of glucocorticoids and stroke (chapter 2)

#	Criteria	Number (%) of papers meeting criteria
1	Title and abstract	18 (38)
Introduction		
2	Background/rationale	48(100)
3	Objectives	41 (85)
Methods		
4	Study Design	47 (98)
5	Setting	41 (81)
6	Participants	43 (90)
7	Variables	44 (92)
8	Data sources/measurement	48 (100)
9	Bias	36 (75)
10	Study size	1 (2)
11	Quantitative Variables	42 (88)
12	Statistical methods	42 (88)
Results		
13	Participants	40 (83)
14	Descriptive data	40 (83)
15	Outcome data	46 (96)
16	Main results	41 (85)
17	Other analyses	42 (88)
Discussion		
18	Key results	47 (98)
19	Limitations	23 (48)
20	Interpretation	46 (96)
21	Generalizability	36 (75)
Other information		
22	Funding	24 (50)

Appendix 3: User group briefing information sheet

User Group

Firstly thank you for allowing me to attend your group to speak with you about my research study.
There had been increasing interest in recent years in involving service users in research design for a number of reasons outlined below:

- People who use services are able to offer different perspectives
- People who use services can help to ensure that issues identified and prioritised are important to them and therefore to health care, public health and social care services as a whole.
- Public involvement can help to ensure that money and resources aren't wasted on research that has little or no relevance.
- Service users can help to ensure that research doesn't just measure outcomes that are identified and considered important by professionals
- Service users have been found to be a good way of disseminating important research results into the public domain

The main aim of this discussion is so to find out how you, as service users and stroke survivors, view the study and in particular the information I am providing for potential participants in the study, but also the way the study is conducted. This is really important, as you have a unique insight into how potential participants will be feeling about research after they have had a stroke and how I can ensure my study is well designed to minimise burden to participants.

Study Details

Study title: Delirium and long-term cognitive impairment after stroke: the role of the HPA axis

Description of the study: People who have had a stroke sometimes have problems with thinking and alertness. This is called delirium. A hormone called cortisol is thought to be important in this process. Cortisol levels can be measured from a small sample of saliva. This study will find out whether

cortisol is related to problems with thinking in the first weeks after a stroke. We also want to find out whether cortisol levels are abnormal in the longer term, and whether this is related to longer term problems with memory and thinking.

Recruitment: We will be recruiting people from the stroke unit, in the first few days after their stroke and will plan to follow them up for one year. We are planning to include those who are not able to give consent for themselves, buy asking their nearest relative for consent. Details of when follow up will be done and what this will involve can be found in the patient information sheet.

Appendix 4: Patient and proxy information sheets and consent forms

Patient information sheet

Study Title: Delirium and long-term cognitive impairment after stroke

You are being asked to consider taking part in a research study. Before you decide whether or not to take part it is important that you understand what it will involve and why the research is being done.

This information sheet tells you about the purpose and conduct of the study and what will happen if you take part

Please ask us if there is anything that is not clear or if you would like to know more. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

People who have had a stroke sometimes have problems with thinking and alertness. This is called delirium. A hormone called cortisol is thought to be important in this process. Cortisol levels can be measured from a small sample of saliva. This study will find out whether cortisol is related to problems with thinking in the first weeks after a stroke. We also want to find out whether cortisol levels are abnormal in the longer term, and whether this is related to longer term problems with memory and thinking.

Why have I been chosen?

You have been asked to take part because you have recently had a stroke, are in hospital and over the age of 60.

Do I have to take part?

It is up to you to decide whether or not to take part. You will be given as long as you feel you require to make this decision, up to 24 hours, and will always be given enough time to discuss this with your family should you wish. If you do decide to take part you will be given this information sheet to keep and the Chief Investigator will ask you to sign a consent form. You can decide to stop your involvement at any time. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?
What do I have to do?

Whilst you are in hospital:

The researcher will discuss the study with you in detail and give you the opportunity to ask any questions.

Saliva Samples
The researcher will help you collect a small sample of saliva. This will be done by placing a small cotton bud under your tongue for a couple of minutes. This will be done twice on the first day that you take part (in the morning and early evening). It will be repeated on a further 6 occasions whilst you are in

hospital. If your hospital stay is short, we may ask to visit you at home in the first month after discharge. You will be asked not to drink, eat or smoke for 20 minutes before the sample is taken. The hormone cortisol will be measured in the saliva. The saliva samples will then be analysed for the hormone cortisol and will then be stored for 3 years and may be used for future, ethically approved, studies.

Interview
On the first day you will then be asked some questions to test your memory and alertness. These questions will take about 20 minutes. We will also ask your Next of Kin to complete a questionnaire designed to check how your memory was before the stroke. Then, a shorter set of questions will be asked at each subsequent visit whilst you are in hospital. We will use a tape recorder to record some of your responses. This is to ensure that you are given an accurate score for the tests. The recording will be deleted as soon as we have scored the tests.

Information from your notes
We will obtain information about your stroke from your medical notes and from the ward doctors and nurses.

Brain Scans
When you were admitted to hospital, you had a brain scan. We would like to look at this scan in detail, with a consultant neuroradiologist.

Data Linkage
The National Health Service Central Register contains basic details about everyone born in Scotland and anyone else who is (or has been) on the list of a general practitioner in Scotland. We would like to use information held by the NHS and records maintained by the National Health Service Central Register for Scotland to keep in touch with you and follow your health status in the longer term.

Follow-up at home:

You will be visited in your home 4 months after your stroke, and then again at one year after your stroke. At these visits we will take salivary samples as described previously. We will also test your memory in a little more detail. These tests will take approximately 1 hour in total. If you prefer not to be seen at home, we can arrange for you to be seen in hospital.

Will any genetic tests be done?

No. Genetic testing is not a part of this study

Involvement of the General Practitioner/Family doctor (GP)

Your own general practitioner will be informed about your participation in the study. If you are still in hospital when the interview is conducted, we will write in your medical records that you are taking part.

What are the possible disadvantages and risks of taking part?

There are minimal risks involved in the study, because we are not testing new treatments.

What are the possible benefits of taking part?

With your permission, we give your doctors and nurses the results of the memory tests, because they may find this information helpful.

What happens when the research study stops?

No further involvement is required. We will ask your permission to store your saliva samples in the Clinical Research Facility, Royal Infirmary so that we can consider them for use in future research studies that we may carry out. Any future use of samples would not identify you by name and would require the approval of a Research Ethics Committee for that project.

What if there is a problem?

Complaints:

If you have a concern about any aspect of the study, you should ask to speak with Dr Barugh, Professor MacLullich or Professor Mead who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the complaints procedure in the hospital. Contact details are outlined at the end of this information sheet

Harm:

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal hospital complaints mechanisms will be available to you.

What will happen to the results of the research study?

The results of the study will be published in medical journals and presented at professional conferences. You will not be identified in any publication or presentation. If the results show that delirium and longer term memory problems are related to high levels of cortisol, we will do further research to find out whether treatment to reduce cortisol levels prevents delirium and longer term cognitive impairment after stroke.

Who is organising and funding the research?

The study is being organised by a team of doctors and researchers working in the Royal Infirmary of Edinburgh and is funded by the Dunhill Medical Trust.

Will my taking part in this study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. The information will be stored securely and any identifying information will be removed. Our procedures for handling, processing, storage and destruction of data comply with the

Data Protection Act 1998. The information will be retained for three years and then disposed of securely. You have the right to check the accuracy of data held about you and correct any errors.

The sponsors of the research (University of Edinburgh and NHS Lothian) and regulatory authorities will have access, as necessary, to view the data for monitoring the quality of research. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

Who has reviewed the study?

The study has been approved by the Scotland A Research Ethics Committee.

You will be given a copy of this information sheet and the consent form to keep.

Thank you for considering taking part in this study

Contact details for the Chief Investigator:

Dr Amanda Barugh,

Research Fellow and Honorary Specialty Registrar in Medicine of the Elderly,

Room s1643,

If you wish to make a complaint about the study please contact NHS Lothian:

NHS Lothian Complaints Team,

2nd Floor,

Waverley Gate,

2-4 Waterloo Gate

Relative/proxy information sheet

Study Title: Delirium and long-term cognitive impairment after stroke

You are being asked to consider whether you would give consent for your relative to take part in a research study because your relative is unable to give consent themselves. You should base your decision on what you believe your relative would decide to do, if they were able to make the decision alone. Before you decide whether or not to give consent it is important that you understand what it will involve and why the research is being done.

This information sheet tells you about the purpose and conduct of the study and what will happen if your relative takes part.

Please ask us if there is anything that is not clear or if you would like to know more. Take time to decide whether or not you wish to give consent on your relative's behalf.

What is the purpose of the study?

People who have had a stroke sometimes have problems with thinking and alertness. This is called delirium. A hormone called cortisol is thought to be important in this process and cortisol levels can be measured from a small sample of saliva. This study is to try and find out if cortisol is related to problems with thinking in the first weeks after a stroke, and to find out if problems with cortisol regulation are seen longer term following stroke. We also hope to determine whether high cortisol levels in turn lead to longer term problems with memory and thinking.

Why has my relative been chosen?

Your relative has been asked to take part because they have recently had a stroke, are in hospital and over the age of 60.

Do I have to agree?

It is up to you to decide whether or not to give consent. You will be given as long as you feel you require to make this decision, up to 24 hours, and will be given time to discuss this in detail with your relative. If you do decide to give consent you will be given this information sheet to keep and the Chief Investigator will ask you to sign a consent form. You or your relative can decide to stop involvement at any time. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your relative receives.

What will happen to my relative if they take part?
What will they have to do?

Whilst your relative is in hospital:

The researcher will discuss the study with you and your relative in detail and give you the opportunity to ask any questions

Saliva Samples

Your relative will be asked to provide a small saliva sample, which they will be helped to obtain by the researcher. This will be collected by placing a small cotton bud under their tongue for a couple of minutes. This will be done twice on the first day that they take part (in the morning and early evening) and this process will be repeated on 6 subsequent days over the following month, if they remain an in-patient. If they are discharged more quickly, we may ask to undertake some of this in their own home with both your and your relative's permission. Participants will be asked not to drink, eat or smoke for

20 minutes before the sample is taken. The saliva samples will then be analysed for the hormone cortisol and will then be stored for 3 years and may be used for future, ethically approved, studies.

Interview

On the first day your relative will be asked some questions to test their memory and alertness. These questions should take approximately 20 minutes. We will also ask you to complete a questionnaire designed to check how your relative's memory was before the stroke. Then, your relative will be asked a shorter set of questions at each subsequent visit whilst they are in hospital. We will use a tape recorded to record some of your relative's responses. This is to ensure they are given an accurate score for the tests. The recording will be deleted as soon as we have scored the tests.

From case notes

We will obtain information about how your relative is and the type of stroke they have had from their medical notes and from the ward doctors and nurses.

Brain Scans

Your relative will have had a scan taken of their brain as part of usual medical care. We would like to ask for your permission look at this scan in detail with a consultant neuroradiologist.

Data Linkage

The National Health Service Central Register contains basic details about everyone born in Scotland and anyone else who is (or has been) on the list of a general practitioner in Scotland. We would like to use information held by the NHS and records maintained by the National Health Service Central Register for Scotland to keep in touch with you and your relative and follow their health status in the longer term.

Follow-up at home:

Your relative will be visited in their home 4 months after their stroke and then again one year after their stroke. At these visits we will take further saliva samples in the same way as described previously and will perform more detailed memory tests. These visits will take approximately 1 hour in total. If preferred we can arrange for your relative to be seen at the hospital, rather than at home.

Will any genetic tests be done?

No. Genetic testing is not a part of this study

Involvement of the General Practitioner/Family doctor (GP)

Your relative's own general practitioner will be informed about their participation in the study. If they are still in hospital when the interview is conducted, we will write in their medical records that they are taking part.

What are the possible disadvantages and risks of taking part?

There are minimal risks involved in the study, because we are not testing new treatments.

What are the possible benefits of taking part?

With your permission, we will inform the medical and nursing team responsible for clinical care about the results of the tests. This may add more detailed information than they have already, and it may help your relative for them to know this.

What happens when the research study stops?

No further involvement is required. We will ask your permission to store your relative's saliva samples in the Clinical Research Facility, Royal Infirmary so that we can consider them for use in future research studies that we may carry out. Any future use of samples would not identify your relative by name and would require the approval of a Research Ethics Committee for that project.

What if there is a problem?

Complaints:
If you have a concern about any aspect of the study, you should ask to speak with Dr Barugh, Professor MacLullich or Professor Mead who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the complaints procedure in the hospital. Details are included at the end of this information sheet.
Harm:
If your relative is harmed by taking part in this research project, there are no special compensation arrangements. If your relative is harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or how your relative was treated during the course of this study, the normal hospital complaints mechanisms will be available to you.

What will happen to the results of the research study?

The results of the study will be published in medical journals and presented at professional conferences. Your relative will not be identified in any publication or presentation. If the results show that delirium and longer term memory problems are related to high levels of cortisol, we will do further research to find out whether treatment to reduce cortisol levels prevents delirium and longer term cognitive impairment after stroke.

Who is organising and funding the research?

The study is being organised by a team of doctors and researchers working in the Royal Infirmary of Edinburgh and is funded by the Dunhill Medical Trust

Will my relative taking part in this study be kept confidential?

Yes. All information which is collected about your relative during the course of the research will be kept strictly confidential. The information will be stored securely and any identifying information will be removed. The information will be retained for one year and then disposed of securely. Our procedures for handling, processing, storage and destruction of data comply with the Data Protection Act 1998. The information will be retained for three years and then disposed of securely.

The sponsors of the research (University of Edinburgh and NHS Lothian) and regulatory authorities will have access, as necessary, to view the data for monitoring the quality of research. All will have a duty of confidentiality to your relative as a research participant and nothing that could reveal their identity will be disclosed outside the research site.

Who has reviewed the study?

The study has been approved by the Scotland A Research Ethics Committee.

You will be given a copy of this information sheet and the consent form to keep.

Contact details for Chief Investigator:

Dr Amanda Barugh,
Research Fellow and Honorary Specialty Registrar in Medicine of the Elderly,
Room s1643,
Royal Infirmary of Edinburgh,
Little France Crescent,
Edinburgh,
EH16 4SA.
e-mail: A.J.Barugh@sms.ed.ac.uk

If you wish to make a complaint about the study please contact NHS Lothian:

NHS Lothian Complaints Team,
2nd Floor,
Waverley Gate,
2-4 Waterloo Gate
Edinburgh,
EH1 3EG
Tel: 0131 465 5708

CONSENT FORM –Patient (version 1)

Title of Project: Delirium and long-term cognitive impairment after stroke.

Name of Researcher:
Name of Participant:

Please tick box

1. I confirm that I have read and understand the information sheet dated 03/07/2012 (version 1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes, including brain scans, may be looked at by responsible individuals from the University of Edinburgh or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

4. I consent to anonymised information from this study being stored for 3 years and used in future, ethically approved, studies ☐

5. I agree to my general practitioner and members of my health care team being informed of my participation. ☐

6. I agree that my saliva will be sampled for the purpose of measuring cortisol and that saliva will be stored, in anonymised form, for analysis of future, ethically approved, studies. ☐

7. I understand that information held by the NHS and records maintained by the National Health Service Central Register may be used to keep in touch with me and follow up my health status ☐

8. I agree to take part in the above study ☐

Name of Participant	Date	Signature
Person Taking Consent	Date	Signature
Witness (if applicable)	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

CONSENT FORM –Relative/Proxy (version 1)
Title of Project: Delirium and long-term cognitive impairment after stroke.

Name of Researcher:
Name of Participant:

Name of relative or carer (signatory on this form):

Is the signatory the welfare guardian of the study participant? Y / N (please delete as appropriate)
Is there a welfare guardian? Y / N
If not, is the signatory the nearest relative to the study participant? Y / N

Please indicate the degree of kinship to the participant ……..

Please initial box

1. I confirm that I have read and understand the information sheet dated 03/07/2012 (version 1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my relative's participation is voluntary and they may withdraw at any time, without giving any reason, without their medical care or legal rights being affected. ☐

3. I understand that sections of any of my relative's medical notes, including brain scans, may be looked at by responsible individuals from the University of Edinburgh or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my relative's records. ☐

4. I consent to anonymised information from this study being stored for 3 years and used in future, ethically approved, studies ☐

5. I agree to my relative's general practitioner and health care team being informed of their participation. ☐

6. I agree that my relative's saliva will be sampled for the purpose of measuring cortisol and that saliva will be stored, in anonymised form, for analysis of future, ethically approved, studies. ☐

7. I understand that information held by the NHS and records maintained by the National Health Service Central Register may be used to keep in touch with my relative and follow up their health status ☐

8. I agree to my relative taking part in this study ☐

Name	Date	Signature
Person Taking Consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 5: Aphasia friendly information sheet

Memory and alertness after stroke

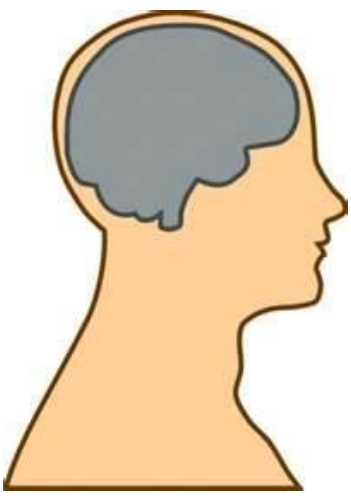
Would you like to take part in research?

Problems with memory and alertness are common after stroke

We are talking to 120 stroke patients

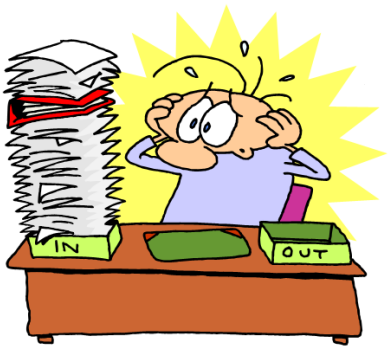
We want to find out……

How many people have problems with alertness after stroke?



How many people have problems with memory after stroke?

How long do these problems last for?



Is the stress hormone called cortisol the cause of these problems?

How can I help?

Visits

In Hospital

6 visits in the month after your stroke

At Home (or in hospital)

One visit at four months and one visit at twelve months after your stroke

What will happen at the visits?

Visits 1 to 6 – In hospital

We interview you and your family

We need information about your stroke

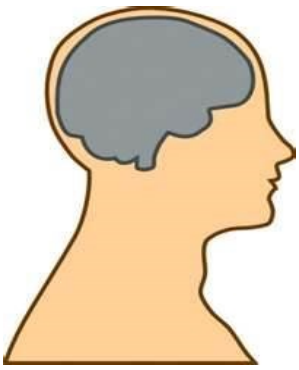
We will read your medical notes

We will take 2 swabs of your saliva to test for cortisol

Visits 7 and 8 –at home (or in hospital)

We interview you

We test your memory in more detail if you are well enough



We take 2 swabs of your saliva to test for cortisol

Everything is PRIVATE

We will not use your name

Talk about this with your FAMILY

Taking part is your choice

It will not affect your healthcare

If you decide to take part

We will tell your GP

We will talk to your FAMILY

You can stop at any time

It is your choice

It is OK to stop

The study results

Will help others who have problems with alertness and memory after stroke

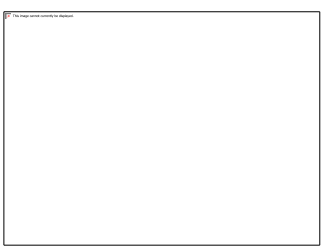
Will be published in medical journals

Funded by

Dunhill Medical Trust

Sponsored by

NHS Lothian



Appendix 6: CT data extraction form

DELIRIUM AFTER STROKE PROJECT P1

CT or MR SCAN READING FORM

Adapted with permission from http://www.sbirc.ed.ac.uk/documents/ctandmr%20reading%20form.pdf

SCAN ID: _____ SCAN DATE: _____

READER ID: _____ READ DATE: _____

SCAN TYPE: CT - CONTRAST: ☐ + CONTRAST: ☐

FORMAT OF CT IMAGES REVIEWED: _____

MR IMAGES REVIEWED: _____

_____

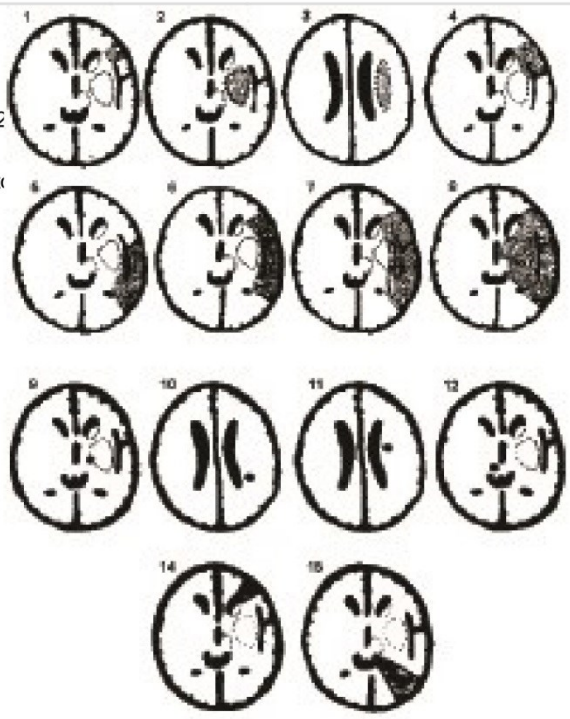
1.	Is the scan completely normal?	Y	N	IF YES, STOP HERE
2.	Is there any sign of acute ischaemic change? (If in doubt, code as acute)	Y	N	IF NO, GO TO Q.10
3.	Which side of the brain is the ischaemia?	R	L	TICK R AND L IF BOTH
4.	Classify signs of ischaemic change in the main lesion.			
a.	Loss of grey / white matter cortex definition	Y	N	
b.	Loss of basal ganglia outline	Y	N	
c.	Definite hypodensity	Y	N	
d.	Mass effect (swelling) present?	Y	N	IF NO, GO TO Q.5
i.	Sulcal effacement?	Y	N	
ii.	Ventricular effacement?	Y	N	
iii.	Midline shift?	Y	N	
iv.	Uncal herniation?	Y	N	

5.	Classify the site and size of ischaemic lesion			Code:

a.	Site				
	M	MCA*	C	Cerebellum*
	AS	Infarct of up to half of ACA territory	SMi	Brainstem - Midbrain*
	AL	Infarct of more than half of ACA territory	SP	Brainstem – Pons
	PS	Infarct of up to half of PCA territory	SMe	Brainstem - Medulla
	PL	Infarct of more than half of PCA territory	CS	Cerebellum and brainstem
	MAS	M+AS*		
	MAL	M+AL*		
	MPS	M+PS*		
	MPL	M+PL*		
	MAP	Infarct of whole MCA, ACA and PCA territories		
	L	Lacune*		
	B	Borderzone*		
		* Code sub-territory sites in "b"		

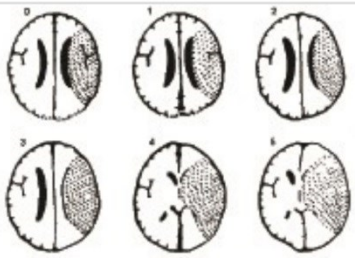
b.	Sub-territory sites

	MCA sub-territory codes
1	small cortical infarct
2	basal ganglia infarct (>2x2x2cm)
3	infarct of WM lateral to the lateral ventricle (>2x2x2
4	infarct of anterior half of peripheral MCA territory
5	infarct of the posterior half of peripheral MCA territ
6	infarct of the whole of peripheral MCA territory
7	6 + infarct of lateral part of basal ganglia
8	infarct of whole of MCA territory
	Lacunar / Borderzone sub-territory codes
9	lacune in internal capsule/lentiform
10	lacune in internal border zone
11	lacune in centrum semiovale
12	lacune in thalamus
13	lacune in brainstem, inc. pons (not shown)
14	anterior (mainly) border zone
15	posterior (mainly) border zone
	Cerebellum sub-territory codes
16	small cortical (not shown)
17	<1/2 hemisphere (medium) (not shown)
18	>1/2 hemisphere (not shown)
	Brainstem sub-territory codes
19	small, i.e.<1/2 medulla (not shown)
20	extensive, i.e. pons + medulla (not shown)MCA*



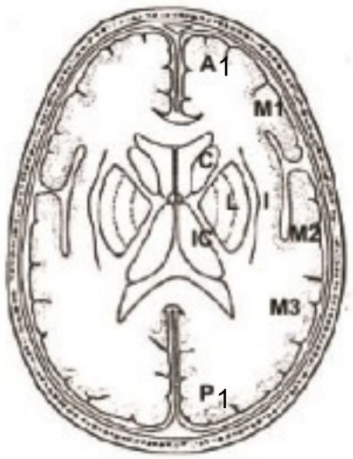
c.	Degrees of mass effect

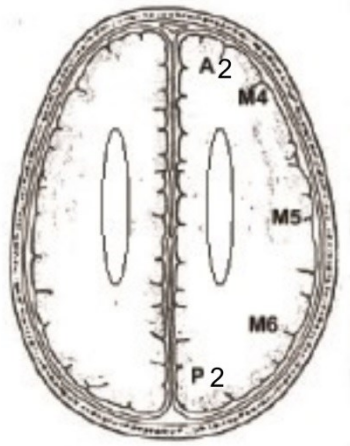
	Mass effect grading
0	**no swelling**
1	effacement of the sulci overlying the infarct
2	1 + minor effacement of adjacent lateral ventricle
3	1 + complete effacement of lateral ventricle
4	1 + effacement of the lateral and third ventricle
5	4 + midline shift away from the side of the ventricle
6	5 + effacement of the basal cisterns



SCAN ID: _____

6.	Does the acute ischaemic change involve more than 1/3 of the MCA territory?	Y	N	
7.	ASPECTS score (modified). Indicate all territories involved:	Normal	Abnormal	





Caudate	N	A	
Lentiform	N	A	
Insula	N	A	
Internal Capsule	N	A	
MCA 1 (M1)	N	A	
MCA 2 (M2)	N	A	
MCA 3 (M3)	N	A	
MCA 4 (M4)	N	A	
MCA 5 (M4)	N	A	
MCA 6 (M6)	N	A	
ACA 1 (A1)	N	A	
ACA 2 (A2)	N	A	
PCA 1 (P1)	N	A	
PCA 2 (P2)	N	A	

8.	Is there a second (discrete) recent ischaemic lesion?	Y	N	IF NO, GO TO Q.10
9.	Describe the second ischaemic lesion:	_____		

Diagrams and score adapted from Lancet 2000;355:1670-1674

X_1	Specify the anatomical location of the infarct:	Right	Left	
	Frontal	R	L	
	Caudate	R	L	
	Lentiform Nucleus	R	L	
	Insula	R	L	
	Internal capsule	R	L	
	Thalamus	R	L	
	Parietal	R	L	
	Occipital	R	L	
	Temporal	R	L	
	Midbrain	R	L	
	Pons	R	L	
	Medulla	R	L	
	Cerebellum	R	L	

10	Is ther a hyperdense artery?	Y	N	IF NO, GO TO Q.12
11	Name the hyperdense artery:	_____		

12	Is there any haemorrhage anywhere?	Y	N	IF NO, GO TO Q.14

13	Identify, rank, and classify the haemorrhage	Rank			<3cm	3-5	5-8	>8cm
a.	Petechial haemorrhage	Rank	Y	N	<3cm	3-5	5-8	>8cm
b.	Significant haemorrhagic transformation of infarct	Rank	Y	N	<3cm	3-5	5-8	>8cm
c.	Parenchymal haematoma (i.e. no infarct visible)	Rank	Y	N	<3cm	3-5	5-8	>8cm
d.	Parenchymal haematoma clearly remote from infarct	Rank	Y	N	<3cm	3-5	5-8	>8cm
e.	Subdural haematoma	Rank	Y	N	<3cm	3-5	5-8	>8cm
f.	Subarachnoid haemorrhage	Rank	Y	N	<3cm	3-5	5-8	>8cm
g.	Extradural haemorrhage	Rank	Y	N	<3cm	3-5	5-8	>8cm

h.	In your opinion, is the haemorrhage likely to have contributed significantly to the burdent of brain damage?	Y	N	

Haematoma with no or only slight mass effect

Haematoma with definite mass effect compressing surrounding tissue

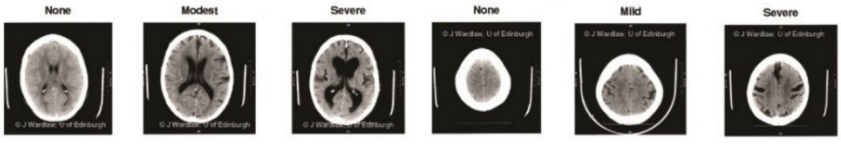
X_2	Specify the anatomical location of any haemorrhage which is separate from the infarct:	Right	Left	
	Frontal	R	L	
	Caudate	R	L	
	Lentiform Nucleus	R	L	
	Insula	R	L	
	Internal capsule	R	L	
	Thalamus	R	L	
	Parietal	R	L	
	Occipital	R	L	
	Temporal	R	L	
	Midbrain	R	L	
	Pons	R	L	
	Medulla	R	L	
	Cerebellum	R	L	

SCAN ID: _____

14	Is there any reduction in brain tissue volume?	Y	N	IF NO, GO TO Q.16
15	Classify the atrophy: None = 0 Mild = 1 Moderate = 2 Severe = 3			
a.	Is the atrophy globally symmetric?	Y	N	IF NO, GO TO Q.15x
	Central atrophy	☐		
	Cortical atrophy	☐		

x.	Classify the asymmetric atrophy:	Central		Cortical		
	Frontal	R	L	R	L	
	If frontal & central, indicate side if caudate contributes to atrophy?	R	L			
	If frontal & central, indicated side if lentiform contributes to atrophic?	R	L			
	Parietal	R	L	R	L	
	Occipital	R	L	R	L	
	Temporal	R	L	R	L	
	If temporal & cortical, indicate side if mesial temporal lobe atrophic in isolation?			R	L	
	Cerebellum	R	L	R	L	
	Brainstem	R	L	R	L	

CORTICAL reduction in brain tissue



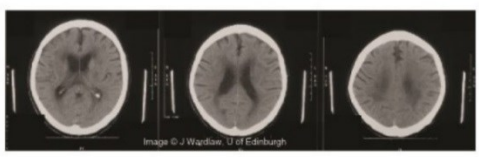
Approach validated in Eur Radiol 2008;19:177-183
Diagrams © J Wardlaw, Univ of Edinburgh

SCAN ID: _____

16	Is there any white matter lucency?	Y	N	IF NO, GO TO Q.18
17	Classify the white matter lucency: None = 0 Mild = 1 Moderate = 2 Severe = 3			
a.	Is the white matter lucency globally symmetric?	Y	N	IF NO, GO TO Q.17x
	Anterior white matter lucency	☐		
	Posterior white matter lucency	☐		

x.	Classify the asymmetric white matter lucency:	Central		Subcortical		
	Frontal	R	L	R	L	
	Parietal	R	L	R	L	
	Occipital	R	L	R	L	
	Temporal	R	L	R	L	
	Cerebellum	R	L	R	L	
	Brainstem	R	L	R	L	

AWM = 1 PWM = 0



AWM = 2 PWM = 1

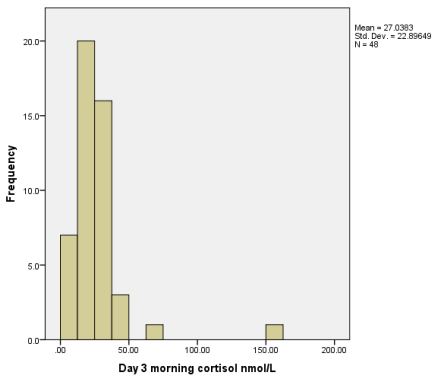
18	Are there any old vascular lesions?	Y	N	IF NO, GO TO Q.20
19	Classify the old vascular lesions:			
a.	Old cortical infarct(s)	Y	N	
b.	Old striatocapsular infarct(s)	Y	N	
c.	Old borderzone infarct(s)	Y	N	
d.	Old lacunar infarct(s)	Y	N	
e.	Old brainstem / cerebellar infarct(s)	Y	N	
f.	Probable old haemorrhage	Y	N	

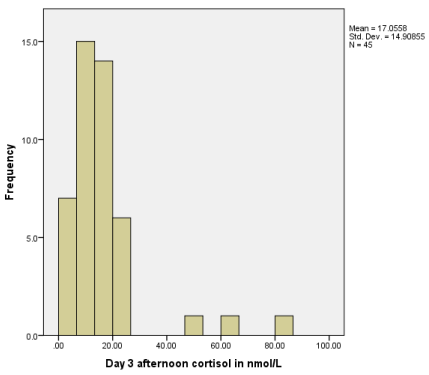
20	Is there a non-stroke lesion which could have accounted for the patient's stroke syndrome?	Y	N	IF NO, GO TO Q.22
21	Classify the non-stroke lesion(s):			
a.	Tumour	Y	N	
b.	Encephalitis	Y	N	
c.	Abscess	Y	N	
d.	Other	Y	N	
	Specify other:	_____		

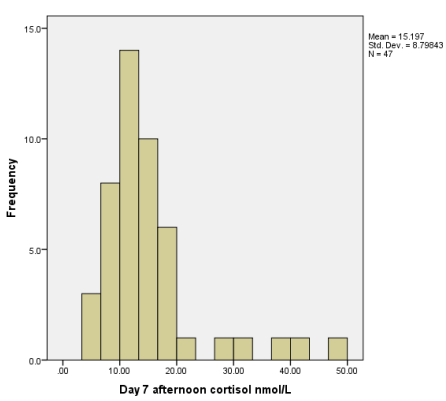
22	Comment	_____ _____ _____

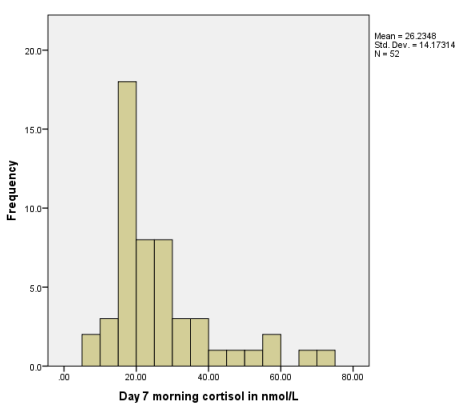
23	Classify scan quality				Good	Mod	Poor

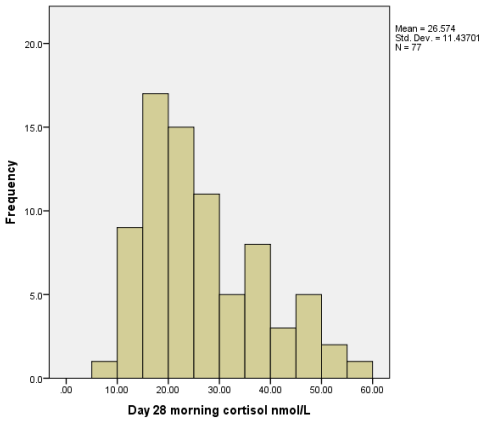
Appendix 7: Histograms of salivary cortisol levels at day 3, day 7, day 28, 4 months and 12 months.

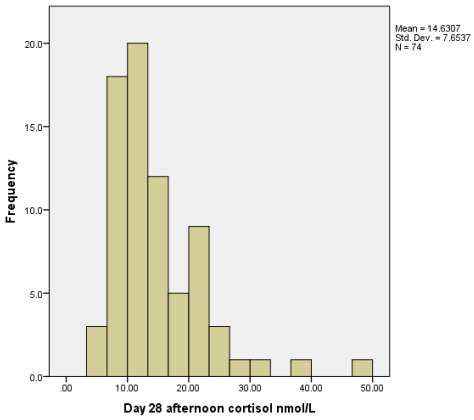


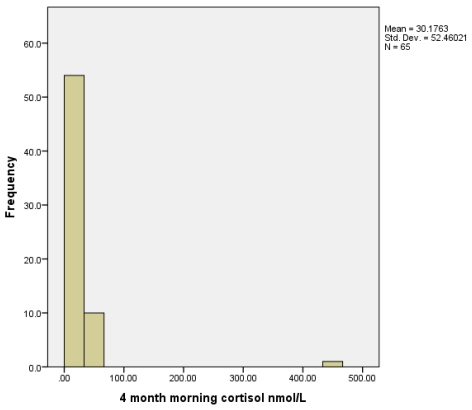


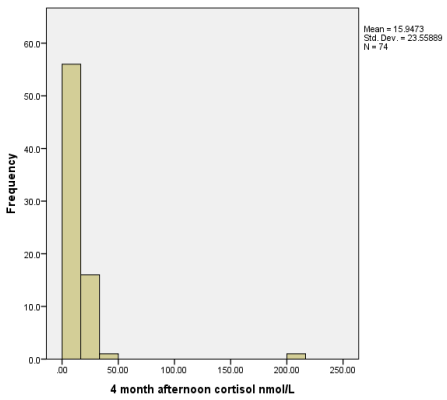


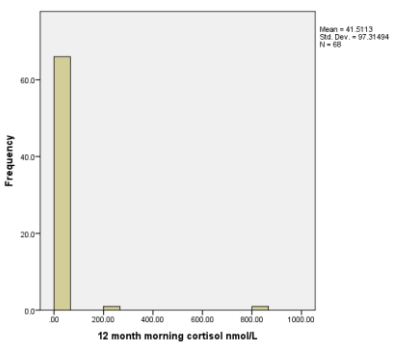


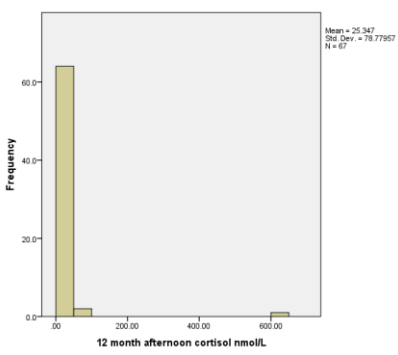

Mean = 26.574
Std. Dev. = 11.43701
N = 77
Frequency
Day 28 morning cortisol nmol/L


Mean = 14.6307
Std. Dev. = 7.6537
N = 74
Frequency
Day 28 afternoon cortisol nmol/L

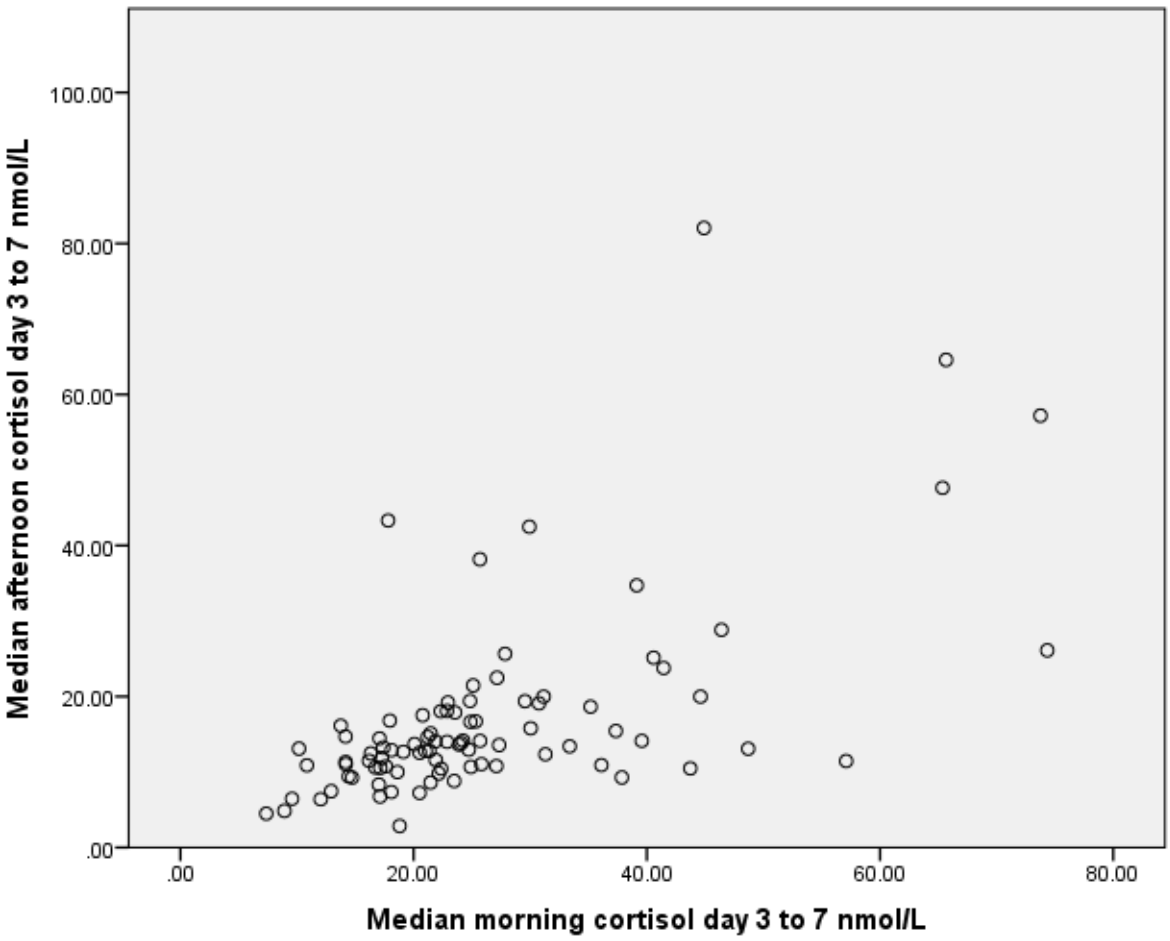

Mean = 30.1763
Std. Dev. = 52.46021
N = 65
Frequency
4 month morning cortisol nmol/L

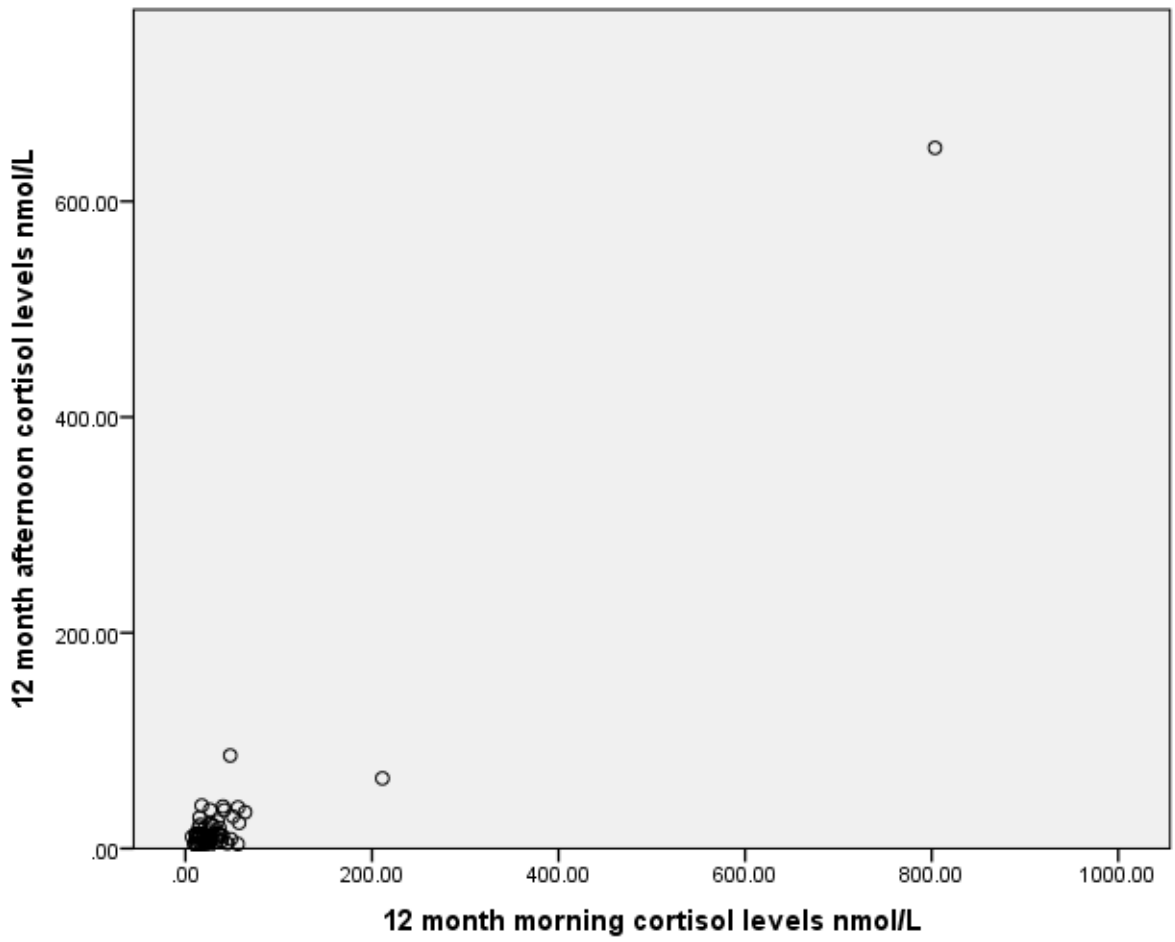

Mean = 15.9473
Std. Dev. = 23.55889
N = 74
Frequency
4 month afternoon cortisol nmol/L


Mean = 41.5113
Std. Dev. = 97.31494
N = 68
Frequency
12 month morning cortisol nmol/L


Mean = 25.347
Std. Dev. = 78.77957
N = 67
Frequency
12 month afternoon cortisol nmol/L

Appendix 8: Scatterplot of median cortisol levels during week 1 and scatterplot of cortisol levels at 12 months




12 month afternoon cortisol levels nmol/L
600.00
400.00
200.00
.00
.00
200.00
400.00
600.00
800.00
1000.00
12 month morning cortisol levels nmol/L

Appendix 9: Permissions from Journal of Neurology and Acta Radiologica

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March 2014, Volume 261, Issue 3, pp 533-545
First online: 30 January 2014
Cortisol levels and the severity and outcomes of acute stroke: a systematic review

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